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PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

OS SONS DO PASTEJO

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Tese apresentada como um dos requisitos à obtenção do Grau de Doutor em Zootecnia
Área de Concentração Plantas Forrageiras

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LIDIANE FONSECA
Engenheira Agrônoma e
Mestre em Zootecnia

TESE

Submetida como parte dos requisitos
para obtenção do Grau de

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“O cientista não é o homem que fornece as verdadeiras respostas; é quem faz as verdadeiras perguntas”. (Claude Lévi-Strauss)

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Os sons do pastejo[1]

Autor: Lidiane Fonseca
Orientador: Paulo César de Faccio Carvalho

Resumo: A capacidade de avaliação do comportamento ingestivo e o consumo de forragem por animais é chave para o entendimento dos processos envolvidos no pastejo. Este trabalho foi conduzido na EEA-UFRGS, com o objetivo de investigar a existência de diferentes tipos de bocados, gerados por diferentes formas de preensão, e também por diferenças estruturais no pasto, que por sua vez geram diferentes características acústicas. Investigou-se a hipótese de que a massa do bocado possa ser estimada por estas características. Os tratamentos consistiram de quatro alturas de pasto e duas espécies forrageiras de hábitos de crescimento contrastantes, quais eram: *Lolium multiflorum* (6, 12 18 e 24 cm) e *Cynodon dactylon* (12, 19, 26 e 33 cm). As variáveis de pasto avaliadas foram: Altura real, Massa de forragem e Densidade do estrato superior do pasto. Estudou-se formas de avaliar a massa do bocado, sendo elas: Método de dupla pesagem, Método de “hand plucking” e Método calculada por meio de fórmulas de um modelo baseado na literatura. Além disso, se investigou o posicionamento de microfones no método acústico, e a capacidade deste método em estimar a massa do bocado e diferenciar tipos de bocados em simples ou compostos. Os resultados demostram que os métodos de avaliar a massa do bocado possuem linearidade entre si, bem como são lineares as relações com as alturas de pasto estudadas. No entanto, a massa do bocado oriunda do método da dupla pesagem e do método calculado apresentaram valores superestimados, o que pode estar relacionado a elevada densidade do pasto, bem como o alto conteúdo de matéria seca. Não foi encontrada diferença nos posicionamentos dos microfones para avaliação das características acústicas dos bocados. O método acústico mostrou-se capaz de discernir entre bocados simples e compostos, porém, em se tratando de tipos de bocados previamente definidos que possuem distintas massas, este método ainda precisa ser aperfeiçoado.

Palavras-chave: Bioacústica, Bocados compostos, Massa do bocados, Tipos de bocados

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[1]Tese de Doutorado em Zootecnia – Plantas Forrageiras, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (98 p.) Agosto de 2014.

The sounds of grazing[1]

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Abstract: The ability to evaluate the ingestive behavior and herbage intake by grazing animals, are the important aspects to the understanding the grazing processes. This work was carried out at EEA-UFRGS, the aim was study bite types generated by different ways of animal harvest the bite.and diferences in sward structure. We hypothesized that this different bite types generate distinct acoustic characteristics and bite mass, which in turn, may be estimated by acoustics characteristics.The treatments were levels of sward surface height (SSH) in two different forage specie: *Lolium multiflorum* (6, 12 18 e 24cm) and *Cynodon dactylon* (12, 19, 26 e 33cm). The sward variables evaluated and discussed on papers in this thesis pasture were: Actual sward surface height, herbage mass and bulk density of superior strata. We had study three ways to estimate the bite mas, which are: Hand plucking, double weighing, and calculated method (based on Baumont et a., 2004 formulas). Furthermore, we study three positions of microfone on animal in bioacustic methodology, besides the ability of this method to estimate the bite mass and discriminate between bite types. The results shown that three methods to evaluate bite mass were linear with each other, and also sith the SSH studied. However, the bite mass from double weighing and Calculated method shown high values, when compared with hand plucking method and others papers, this fact may be due to the high bulk density, as high proportion of dry matter. Was not found differences in microfone position to discriminate different bite types. The acoustic method was able to discriminate single bites and chew bites, however, to the bite types previously defined, which have different bite mass, this methodo still needs to be improved.

Key words: Bioacustic, Bite mass, Bite type, Chew-bites.

[1]Doctor Scintist in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil (98 p.) August, 2014.

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Abreviatura	Descrição
AIC	Akaike information criterion
b	Bite
BA	Bite area
BD	Bite depth
BkD	Bulk Density
BM	Bite mass
BW	Body weight
cb	Chew bite
cm	Centímetro
cm	Centímetros
cm^2	Centímetros quadrados
d	Day
DA	Dental arcade breadth
dB	Decibel
DM	Dry matter
DM	Dry matter
DMI	Dry matter intake
Et al.	Et alii é uma expressão latina que significa "e outros"
Etc.	Et cetera (e as demais coisas)
Fig.	Figura/figure
Freq	Frequência
g	Grama (medida)
h	Horas
ha	Hectare
HM	Herbage mass
IGER	Institute of Grassland and Environmental Research
kg	Kilograma
kHz	Quilohertz
LW	Live weight
m^2	Metros quadrados
m^3	Metros cúbicos
MB	Massa de bocado
MF	Massa de forragem
mg	Miligrama
min	Minutos
mo	Month
MS	Matéria seca
PV	Peso vivo
RMSE	Root mean square error
SSH	Sward surface height
STIR	Short term intake rate
WAV	Wave form audio

1. CAPITULO I

1.1 Introdução

1.2 Modelo Conceitual

1.3 Hipótese e Objetivos

1.4 Revisão Bibliográfica

1.1 Introdução

A habilidade em avaliar o comportamento ingestivo e o consumo de forragem por animais é aspecto chave para o entendimento dos processos envolvidos no pastejo, e importante para tomar decisões de manejo (Ungar, 1996). O consumo é o fator mais determinante da produtividade das pastagens, e também uma das mais importantes medidas de impacto dos animais nos ecossistemas pastoris. Não obstante, sua medição é "a caixa preta" da investigação em pastagens. Os métodos para avaliar o comportamento e estimar o consumo são desafiadores quando realizados no ambiente pastoril e, na maior parte dos casos, caros e imprecisos. Nesse sentido a massa do bocado entra como variável determinante a ser investigada, visto que é a variável central do consumo de forragem. Segundo Laca e Ortega (1995), o bocado pode ser considerado como o átomo do pastejo, o que denota a importância em se encontrar metodologias para avaliar esta variável, pivotal em diversos estudos de comportamento ingestivo, consumo e manejo do pasto.

Novas tecnologias e avanços em métodos analíticos têm procurado incrementar a capacidade da pesquisa em coletar dados relativos ao comportamento ingestivo e o consumo de forragem por animais em pastejo (Carvalho et. al., 2009). Dentre as recentes inovações nesse tema, o método acústico tem sido proposto por ser método não invasivo, de baixo custo e que possibilita identificar as atividades dos ruminantes de forma contínua, sem afetar o comportamento do animal. Tal método já foi proposto em considerável número de publicações (e.g., Laca & Wallis DeVries, 2000; Ungar & Rutter, 2006; Galli et al., 2006; Milone et al., 2009; Clapham et al., 2011; Galli et al., 2011; Da Trindade et al., 2011; Nadin et al., 2012).

Neste contexto, esta tese é composta por quatro capítulos. O Capítulo I traz uma revisão bibliográfica sobre os assuntos tratados no decorrer do trabalho. No Capítulo II apresenta-se um artigo onde se aborda três formas de avaliar a massa do bocado de animais em pastejo, e também é abordado a possibilidade de uso de variáveis acústicas para tal finalidade. O artigo do Capítulo III versa sobre as características acústicas de tipos de bocados (tipos definidos previamente pelo avaliador e bocados simples ou compostos), se discutindo acerca da identificação destes tipos de bocados de acordo com três locais de posicionamento do microfone no animal. Por fim, no Capítulo IV, são apresentadas as considerações finais, onde são apresentadas as conclusões gerais do trabalho, juntamente com as perspectivas futuras da técnica da bioacústica.

1.2 Modelo Conceitual

O modelo conceitual apresentado na Figura 1 trata das relações envolvidas no presente trabalho. Como variável central do modelo está a produção animal a pasto. A massa do bocado (MB) aparece como a variável que será tratada na presente tese e influencia a produção animal, visto que é a principal determinante da taxa de ingestão. A taxa de ingestão, por sua vez, é a variável que afeta de forma peremptória o consumo diário de forragem. Quando em situação de baixa taxa de ingestão, os animais aumentam a duração da refeição, na tentativa de manter o consumo diário de forragem aumentando, dessa forma, o tempo de pastejo.

A MB é influenciada não apenas pela espécie vegetal e pelo animal que está pastando, mas também é fortemente influenciada pela altura do pasto. A altura do pasto, por sua vez, é a forma como o animal percebe a estrutura do pasto. Consequentemente, diferentes alturas acarretam distintas estruturas de pasto, que promovem diferentes MB.

Neste trabalho, diferentes tipos de bocados tomados pelos animais serão abordados. Estes tipos de bocados diferem de acordo com a massa do bocado, que como descrito acima, responde à altura do pasto, à espécie vegetal e ao animal.

Outro ponto a ser tratado são as características acústicas dos bocados, as quais são influenciadas pela massa do bocado e todas as variáveis que a influenciam, assim como os tipos de bocados a serem tratados nesta tese.

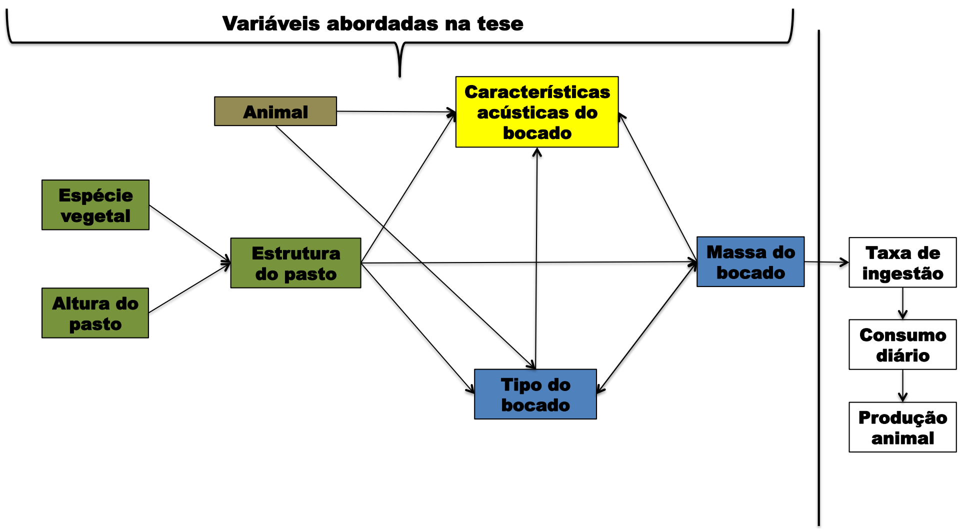


Figura 1: Modelo conceitual sobre como variáveis relacionadas ao animal e ao pasto definem diferentes tipos de bocado e suas características acústicas.

1.3 Hipóteses e Objetivos

1.3.1 Hipótese*:*

- Existem diferentes tipos de bocados, gerados por diferentes formas de preensão e por diferenças estruturais no pasto. Essas diferenças se refletem em diferentes características acústicas, que poderiam ser utilizadas para estimar a massa de cada bocado.

- O posicionamento de um microfone, ou de um conjunto de microfones no animal, afetaria a aferição das características acústicas gravadas.

1.3.2 Objetivos*:*

- Definir as características acústicas dos diferentes tipos de bocado;
- Estimar a massa do bocado por meio de várias metodologias, para validar a estimação via características acústicas;
- Avaliar se o método acústico é capaz de predizer a massa do bocado;
- Estudar o posicionamento posicionamento dos microfones que proporcionem melhor qualidade nas gravações acústicas;
- Avaliar se o método acústico é capaz de diferenciar tipos de bocados e bocados compostos.

1.4 Revisão Bibliográfica

1.4.1 Definições

Bioacústica é a combinação de duas áreas de estudo: A acústica e a biologia. Ela normalmente se refere a produção, dispercao e recepção de som dos animais. As métricas utilizadas na bioacústica para classificar e quantificar processos sonoros (no caso do presente estudo os processos ingestivos), são a frequência, amplitude e envelope. A frequência é o número de ondas sonoras que passa por um determinado a cada segundo. A amplitude corresponde à quantidade de energia em cada onda sonora. O envelope por sua vez, é a forma como o som se inicia, se mantém e termina ao longo do tempo.

(a)

(b)

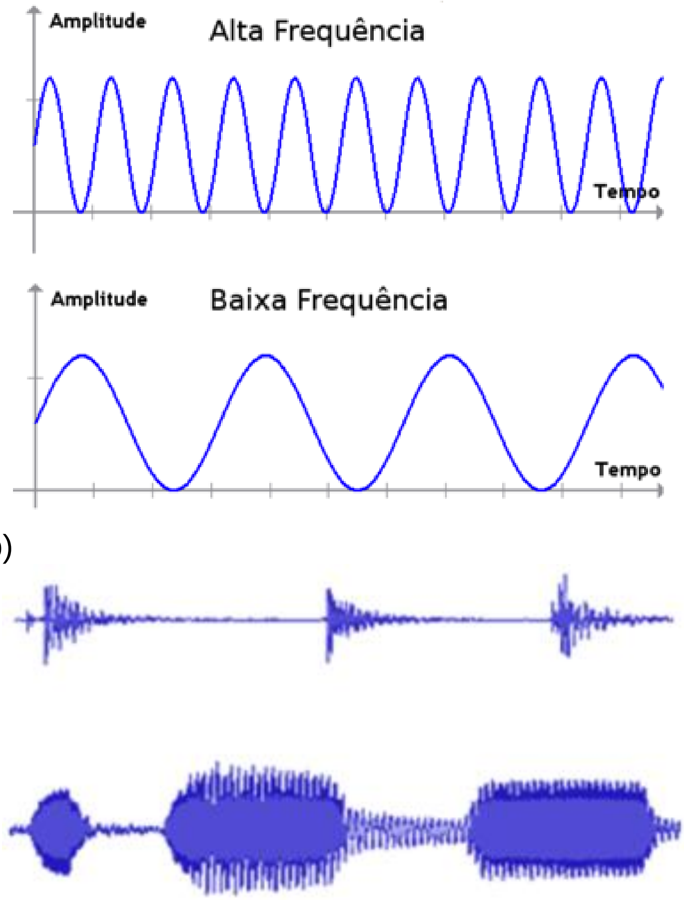


Figura 2 representação das métricas sonoras (a) Amplitude e frequência e (b) envelope.

1.4.2 O que é a bioacústica e quais os seus usos atuais?

Bioacústica, no caso do presente estudo, refere-se ao estudo dos sons emitidos pelos animais. Ela é a junção de duas áreas de estudo, a acústica somada a biologia. Esta técnica teve seus primeiros passos após avanços tecnológicos decorrentes da segunda guerra mundial (Vielliard & Silva, 2011). Desde então, é uma metodologia muito útil tanto na ecologia como na zootecnia, pois é bastante utilizada para monitoramento de várias espécies animais selvagens ou domesticados, além de ser imprescindível para caracterização de espécies (Vielliard, 1993).

1.4.2.1 Usos da bioacústica na ecologia

A bioacústica permite a investigação de áreas que contêm populações de determinadas espécies além de investigar períodos de tempo que contêm atividades biológicas importantes, tais como o acasalamento, migração e forrageamento. Registos acústicos podem ser utilizados por biólogos de conservação visando detectar a presença de espécies de animais ameaçadas de extinção ou raras (Blumstein et al., 2011).

O monitoramento acústico têm sido amplamente utilizados para monitorar a vida marinha em todo o mundo por várias décadas (e.g. Richardson et al., 1995). O seu potencial para a monitorização dos animais em ambientes terrestres, no entanto, só foi identificada mais recentemente (e.g. Oliveira & Ades, 2004; Gross-Louis et al., 2008; Monticelli & Ades, 2011). Os ornitólogos foram os primeiros cientistas à fazerem uso da bioacústica fora da água (e.g. Vielliard, 1989; Vielliard, 1993; Vielliard & Siva, 2011). Esta técnica não é apenas a coleta de informações sobre a presença de espécies. Os recentes avanços na bioacústica mostram que ela pode ajudar a estimar a riqueza de espécies e densidade de espécies, informar os padrões de atividade dos animais, a fenologia reprodutiva, ou entender as interações entre os indivíduos. Essa informação pode então ser usada para acompanhar os efeitos das alterações climáticas, fragmentação de habitats ou as perturbações antrópicas sobre o comportamento, a distribuição ou a densidade de vida selvagem (Blumstein et al., 2011). Considerando a recente necessidade de enfrentar a mudanças ambientais e suas conseqüências sobre a biodiversidade a bioacústica na ecologia desponta como uma importante ferramenta para monitoramento e pesquisas nessa área (Blumstein et al., 2011; Pereira & Cooper, 2006).

1.4.2.2 Usos da bioacústica na zootecnia

Na zootecnia a bioacústica é amplamente utilizada como ferramenta para avaliar o bem estar de animal através da vocalização, assim como forma de identificação de possíveis fatores de estresse de varias espécies de animais como: suínos, ovelhas, bovinos de corte e leite, aves, entre outros (e.g. McCowan et al., 2002; Manteuffel et al., 2004; Gradin, 1998; 2001; Nääs et al., 2008).

No entanto no presente trabalho, assim como em diversos outros que serão apresentados no decorrer desta revisão e na discussão dos artigos desta tese, tratam o uso da bioacústica como identificação de movimentos mandibulares. Intenta-se a aplicação desta técnica para identificação dos sons que os herbívoros domesticos emitem ao pastejar, e que nos são úteis para avaliação do comportamento ingestivo e no consumo pelos animais em pastejo.

1.4.3 Porquê estudar a bioacústica no comportamento ingestivo?

Medidas de comportamento ingestivo podem ser realizadas por observação direta (Mezzalira et al., 2011). Mas são avaliações exaustivas mesmo para observadores treinados, dificultando a coleta de dados e a precisão das observações por longos períodos, sobretudo durante o período noturno. Equipamentos e sistemas automatizados desde longa data têm sido empregados no monitoramento do comportamento ingestivo, entre eles o Ethosys, Medilog, Vibracorders, APEC, acelerômetros, mas o de uso mais frequente é o IGER Behaviour Recorder (Carvalho et al., 2007). Esse aparelho registra movimentos mandibulares e um software (Graze Analysis Program) os distingue em bocados e movimentos mandibulares de não-bocado, bem como o tempo efetivo de alimentação e ruminação (Rutter, 2000). Os movimentos mandibulares podem ser identificados e classificados como bocados e não-bocados de acordo com critérios de amplitude e frequência especificados pelo usuário do software (Ungar & Rutter, 2006). Os registros podem ser observados na escala de segundos e ainda são capazes de fornecer o tempo de pastejo, ruminação e outras atividades, dentre outras importante informações acerca do comportamento ingestivo dos animais.

A bioacústica, por sua vez, possibilita a identificação das atividades dos ruminantes de forma contínua, sem afetar o comportamento dos animais, além de ser um método não invasivo e com baixo custo para aquisição dos equipamentos. O princípio está no fato das atividades dos animais apresentarem características acústicas que potencialmente permitem discriminá-las (e.g., Laca & Wallis DeVries, 2000; Ungar & Rutter, 2006; Galli et al., 2006; Milone et al., 2009; Clapham et al., 2011; Galli et al., 2011; Da Trindade et al., 2011; Nadin et al., 2012).

Uma importante vantagem da bioacústica em relação às demais técnicas já utilizadas para avaliar o comportamento ingestivo, é a possibilidade de discriminação de movimentos mandibulares compostos, onde a forragem ja colhida é mastigada pelo animal, ao mesmo tempo em que o mesmo colhe outro bocado (Penning et al., 1991; Laca et al., 1992, 1994).

Além da avaliação do comportamento ingestivo supracitado, a bioacústica pode ser útil para a medicão da massa do bocado e consequentemente do consumo pelos animais em pastejo. Neste aspecto de medição do consumo, existem diversas metodologias sendo utilizadas, tais como: Marcadores internos como N-alcanos (Mayes et al. 1986; Dove & Mayes 1996; Mayes & Dove, 2000; Gordon, 1995), utilização de animais fistulados, utilização da técnica da dupla pesagem aliado com aparelhos que registram os bocados e tempo de alimentação (Penning & Rutter, 2004).

1.4.4 Métodos utilizados para avaliar o comportamento ingestivo de animais em pastejo e estimar a massa do bocado

Desde publicações como a de Penning et al. (1984), que publicou detalhes dobre um sistema automático de avaliação do comportamento ingestivo de animais em pastejo, tem se publicado inúmeros trabalhos neste contexto, com diversas metodologias utilizadas. Brun et al. (1984) desenvolveu um sistema baseado em um sensor similar ao de Penning et al. (1984), este sensor consistia em um sensor ligado à mandibular do animal, este sistema era potencialmente mais confiável do que o utilizado por Penning. No entanto este sistema apenas identificava se o animal estava pastejando ou não em intervalos de 2,5 s, e com os dados era possivel obter apenas o tempo de pastejo e ruminação, sem informações acerca dos movimentos madibulares.

Outra ideia similar com a de Penning foram utilizada nos trabalhos de Matsui and Okubo (1991, 1993), os quais desenvolveram sistemas de microfones conectados à gravadores. Este, por sua vez, fornecia os movimentos mandibulares e o número de pausas maiores do que 3 s entre estes movimentos, isto por minuto. Estes dados forneciam o tempo de pastejo e ruminação, o número de movimentos mandibulares totais, e o número de boli durante a ruminação. No entanto, este sistema, não discriminava entre movimentos de bocados e mastigações, e consequentemente não permitia obter informações confiável acerca da massa do bocado. Outro sistema com microfone foi desenvolvido por Luginbuhl et al. (1991). Este, por sua vez, fornecia o número de bocados, escutando o som (ou seja análise “manual”). Os movimentos mandibulares totais, neste sistema, era obtido por um buçal elastic, em que os contatos elétricos incorporados se quebravam toda vez que o animal abria a mandibula (Luginbuhl et al., 1987). Neste sistema a discriminação entre pastejo ou ruminação era baseado na posição da cabeça do animal (fornecida por um interruptor na cabeça), se a cabeça do animal estivesse para baixo indicava que era movimento de pastejo se estivesse levantada era movimento de ruminação. No entanto, este método foi muito criticado, pois a exata posição do interruptor não era confiável, podendo gerar muitos erros nas medicos (Penning, 1983).

Um método acústico inicial, similar o atual utilizado no presente trabalho, foi proposto por Alkon et al. (1989) para avaliar o comportamento de porcos-espinho (Hystrix indica). Posteriormente, Laca et al. (1994) constataram em bovinos que bocados e mastigações poderiam ser facilmente identificados e contados pela inspeção de registros acústicos, ao invés da observação direta.

Além das importante informaçoes sobre os movimentos mandibulares, bem como se estes são de bocados, mastigação ou compostos, a investigação nesta area de estudo necessita de metodologias capazes de avaliar a massa do bocado e, mais ainda, a possibilidade de estimar o consume em pastejo de forma confiável. Neste sentido têm-se utilizado metodologias como a da dupla pesagem aliada com a contagem (automatizada ou manual) dos bocados. (e.g. Fonseca et al., 2013; Prache & Damasceno, 2006)

IGER aliado com a técnica da dupla pesagem: E um método de bastante acurácea, no entanto pode ser bastante trabalhoso, e a obtenção dos resultados e restringidas à experimentos de curta duração, impossibilitando a aplicação em escalas diárias.

Grid – Método de hand-plucking: Previamente à realização das avaliações e de acordo com a estrutura do pasto avaliado é definido um “grid” de bocados, que consistem em códigos que caracterizam bocados tomados pelos animais. Este método, pdoe ser muito eficiente, no entanto requer observadores exaustivamente treinados, que irão contabilizar e caracterizar de acordo com o o”grid” todos os bocados tomados pelo animal em um período de tempo. Este método possibilita a estimativa da massa do bocado, e a variação desta, no entanto, não permite a contagem total dos movimentos mandibulares, e nao fornece tempo de pastejo, ruminação, e embora permita estimar o consumo, não pode ser considerado altamente confiável na pesquisa com este fim (para melhor descrição da técnica vide: Bonnet et al., 2011)

Novas técnicas para medir a massa do bocado são, urgentemente, necessárias, ja que muitas vezes, medições alternativas de massa do bocado, como através de estimativas por meio da biomassa ou da altura (Bergman et al. 2000), podem ser requeridas para muitas aplicações no campo.

Segundo Laca & Wallis DeVries (2000), a gravação acústica contém alta riqueza de informações que podem ser reunidas de maneira que não interfira no comportamento de pastejo e que pode prestar-se a análise automatizada e pouco subjetiva (Milone et al., 2009; Clapham et al., 2011). Além disso, uma vantagem importante da abordagem acústica é que permite a contagem de mastigações e bocados, assim como de movimentos compostos de mastigação+bocado (Ungar & Rutter, 2006). Neste contexo a maioria das técnicas ja utilizadas e consolidadas para avaliar o comportamento ingestivo não consegue discriminar estes movimentos compostos de mastigação, manipulação e bocados (Penning et al., 1991). Movimentos mandibulares compostos, nomeados de mastigação+bocado (chew-bite) já foram detectados em bovinos por Laca & Wallis DeVries (2000) foram acusticamente confirmados em ovinos e seu espectro reconhecido (Milone et al., 2009).

Além destes fatores supracitados, alguns estudos promissores indicam a possibilidade de utilizar a técnica acústica para quantificar a ingestão da forragem (Laca & Wallis DeVries, 2000; Galli et al., 2006; Galli et al., 2011), havendo evidências de que esta técnica possa predizer também o tipo de espécie vegetal ingerida pelo animal, bem como sua digestibilidade (Carvalho et al., 2009).

Embora o método acústico revele ser altamente promissor para registrar e quantificar eventos de ingestão, a classificação manual desses eventos em softwares de áudio é difícil, demorado e necessita de automação (Ungar & Rutter, 2006). Além disso, segundo Clapham et al. (2011), é necessário utilizar gravadores e microfones que captem e registrem som em ampla frequência (0-22kHz) e que os armazenem no formato WAV (wave form audio) para que facilite a diferenciação entre os movimentos mandibulares de bocados e mastigações.

2. CAPITULO II

Methods to determine the bite mass its relationship with sward surface height and the estimation of bite mass from sounds variables [1]

[1] Artigo elaborado de acordo com as normas da revista Grassland Science (Apêndice 1)

Methods to determine the bite mass its relationship with sward surface height and the estimation of bite mass from sounds variables

Abstract: Several problems related to the estimation of herbage intake are associated with the difficulty in estimating the bite mass (BM). New technologies and advances in analytical methods have sought to increase research capacity in collecting data on the herbage intake by grazing animals. In this sense, the bioacustic appears as a promising method. This study aimed to compare methods to measure bite mass of heifers in grazing condition. Moreover, we tested the estimation of BM from sound's variables of the bites. The experiment was carried out at the experimental station of Federal University of Rio Grande do Sul (EEA-UFRGS). Two experiments were conducted, one using *Lolium multiflorum* and the other with *Cynodon dactylon* microswards, grazed by four Angus × Brahman beef heifers (60 ± 2 month old, 395 ± 6.7 kg BW). The treatments were four levels of sward surface heights (SSH) (i.e: *Lolium multiflorum* 6, 12 18 and 24cm, *Cynodon dactylon*, 12, 19, 26 and 33cm). A *sward stick* was used to determine the SSH, 10 points per microsward were measured in each grazing session. The microsward, used in the grazing session, was placed on the floor of the arena so that the soil surface was approximately 15 cm above the level at which the animals were standing. The microsward was offered to each animal until it started to take bites in the second grazing horizon, and each bite was described according to a predefined type. Bite mass was estimated by three methods: corrected microsward mass loss, product of bite dimensions and sward bulk density and simulated bites (hand plucking)., and for *C. dactylon* the bite mass was estimated by the acoustic characteristics. Bite masses calculated by all methods were linearly related to SSH, with the best relationship being for the bite dimension method. BM estimated by the three methods presented linear relationship among them. Although was possible to estimate the BM from sound variables, i.e. bioacustic, the method has to be further improved to estimate BM and herbage intake in grazing condition.

Keywords: Bite types, hand-plucking, calculated bite mass, double weight method.

Introduction

Herbage intake is a central variable to understand and predict the animal performance in grazing studies (Illius and Gordon 1987; Burns et al. 1994). Bite mass, in turn, is assumed to be a determinant for the daily intake and consequently, for the performance of grazing animals (Gordon and Benvenutti, 2006; Fonseca et al., 2013). At the instantaneous scale, intake provides a direct measurement of the animal's ability to grazing. However, the measurement of herbage intake, particularly in a free ranging condition, is still a challenge.

Several problems related to the estimation of herbage intake are associated to the difficulty in estimate the bite mass. There are only a few methods that can be used to estimate bite mass in a free ranging herbivores, such as: Esophageal fistulation, which is considered the method that gives the most accurate results (Stobbs, 1973), however, according to Casey et al. (2004) only a few grassland research facilities have access to oesophageally fistulated animals, so alternatives need to be evaluated. Double weighing technique is another thechnique which is used in several works to estimate bite mass (e.g. Fonseca et al., 2013; Mezzalira et al., 2014; Prache & Damasceno, 2006) is a good way, however is difficult to use with free grazing animals. The hand plucking method, which is developed in different ways for some studies (e.g. Agreil and Meuret, 2004; Bonnet et al., 2011; Wallis de Vries, 1995) but in outline consists of a collect manually of the plant material simulating the bite mass. New technologies and advances in analytical methods have sought to increase research capacity in collecting data on the herbage intake by grazing animals (Carvalho et. al., 2009), among them the bioacustic appears as a promising method. Therefore, this study aimed to i) compare methods to measure the bite mass in grazing condition and ii) to estimate the BM from sound variables of the bites.

Materials and methods

Animals and Procedures

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Federal University of Rio Grande do Sul and were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Experimental site, animals and treatments

The experiment was carried out at experimental station of Federal University of Rio Grande do Sul (EEA-UFRGS). Each forage species was planted in 84 boxes (microswards) each measuring 0.6 m X 0.4 m, 64 of these were used for the grazing sessions and the remaining were used to train the animals. *Lolium multiflorum* swards were sown on June 2012, with a sowing density of 30 kg/ha. At sowing, the boxes were fertilized with 20 kg/ha of N and 100 kg/ha of P2O5 and K2O followed by 200 kg/ha of N 20 d later. *Cynodon dactylon* swards were planted on November 2013 with, approximately 8 seedlings per box. All grazing sessions (described below) were performed in the afternoon in both experiments.

Four Angus × Brahman beef heifers (60 ± 2 month old, 395 ± 6.7 kg BW) were used. Thirty days before the experimental procedure, heifers and evaluators were trained to the experimental protocol with the remaining microswards, with the same sward surface height (SSH) of the experiment. Heifers were not fasted before the grazing sessions, because this may increase intake rates (Gregorini et al., 2009a).

The treatments consisted of two forage species and four levels of sward surface heights (i.e: experiment 1: *Lolium multiflorum* 6, 12 18 and 24cm, experiment 2: *Cynodon dactylon*, 12, 19, 26 and 33cm). To insure that the structure was properly, each forage species in the microsward was cut at 50% of the SSH, always that reached the SSH pre-set for each treatment.

The treatments were randomly applied in a 4 × 4 Latin square design, with four tratments of SSH for each forage specie and four animals, each latin square was replicated four times. The animals were observed in random order, and the sequence of treatments was randomized within animal with the restriction that each animal received a different sequence.

Sward measurements

A *sward stick* was used to determine the SSH, 10 points were measured per microsward in each grazing session. To determine the herbage mass two samples were cut and divided into top 50% and a bottom 50% of the SSH, in two microswards in each treatment, using a quadrat of 0.01 m^2.

All herbage samples were oven dried (55°C over a period of at least 72 h). A digital scale (model Bel Mark 720, 0.001 g of division) was used to weigh the herbage samples.

Grazing sessions

At the morning before the grazing sessions were performed, the animal remained in a paddock with the same forage species used in each experiment. Before the grazing sessions for *C. dactylon* microswards heifers were fitted with microphones and recorders.

The microsward, used in the grazing session, was placed on the floor, so that the soil surface was approximately 15 cm above the level at which the animals were standing. The microsward was offered to each animal and remained until he started to take bites in the second grazing horizon, and each bite was described according to a type predefined, these types consisting in a code which were used to estimate the bite mass by the hand plucking method (described below).

Bite mass estimations

Double weight method

Immediately before and after each grazing session, the microsward used for grazing was weighed to an accuracy of ± 5g with a weighing platform (model Bel Mark KW, 60 kg of the capacity) in a windproof area to measure the herbage dry matter intake (DMI).

The average bite mass (BM) from the microswards weights was calculated based on the number of bites taken in each grazing session and on the corrected weight change of the grazing session in microswards. The herbage DMI was corrected for the herbage dry matter (DM) content in both experiments. The DM content was estimated by the samples used to determine bite mass of hand plucking method (described below), and the weighted average was used according to the bite types taken in each grazing session.

A control microsward of the same species of the grazing session was weighed directly before and after each grazing session to correct for evapotranspiration losses during the grazing session.

Bite dimensions method

To BM calculated, there was considered that BM is product of bite area (BA), bite depth (BD), and bulk density (BkD) of the grazed stratum:

BM = BA * (SSH/2) * BkD (eq. 1)

Where: BA is bite area (cm^2), SSH represents the Sward Surface Height (cm) and BkD is the bulk density of the top half (g/m^3).

Bite depth (SSH/2) was considered as half of SSH (Cangiano *et al.* 2002). Bulk density of the top half herbage mass (BkD; g/m^3) was measured by cutting the herbage in quadrats with $0.01m^2$ in two microswards by treatment.

Empirical components requires fitted parameters, but reflect well-established conceptual models of the grazing process and energy use in ruminants. Bite area (cm^2) was estimated with an empirical model that includes effects of SSH and bulk density (Baumont *et al.* 2004):

BA= 2 * (DA) ^ 2 * (1 + 50 / SSH) ^ (-1) * Exp (-0.3 * (BkD - 1)) (eq. 2)

where: DA is the animal's dental arcade breadth, considered to have an allometric relationship with BA (Illius and Gordon 1987). SSH represents the sward surface height (cm) with an asymptote positive function (Laca *et al.* 1992), and BkD is the bulk density of the top half herbage mass ($g.m^{-3}$) with an exponential negative effect (Laca *et al.* 1992).

Hand plucking method

Was developed a specific coding grids for each specie of sward, to help the observer to categorize bites with regard to its mass. Prior to data collection, the observer was trained for 2 d with each species. Training consisted of first recognizing the bites and classifying them according to the coding grids, and then, collect bite mass, by hand plucking, according to each bite type identified.

Sounds measurements for Cynodon dactylon microswards

Grazing sounds were captured on the system consisted of a digital recorder (Edirol R-09 24-bit recorder, Program Version 1.20, Roland Corporation), and a omni-directional lavalier microphone (Leson ML-70s), that was allocated in the animal forehead, accommodated in a involucre of expanded polystyrene.

Sound data were recorded onto a 2GB SD memory card (Sandisk Extreme III SDHC Card Sandisk Corporation) in the Edirol R-09. All recordings were made at 44.1 kHz sampling rate and 16-bit resolution, providing a nominal 20 kHz recording band, width and 96 dB dynamic range, and stored in the WAV (Waveform Audio) file format. Prior to each recording session, the recorder input level; sampling rate and bit resolution were set; the recorders were secured inside the plastic enclosure.

Statistical analysis

Sounds processing and analysis

Using the Audacity software for Mac (version 2.0.5, http://audacity.sourceforge.net), the stereo files were reduced to monaural files by extracting one channel. Records from different microphones were manually synchronized and labelled. Sounds were simultaneously segmented and classified "by hand" using the software Audacity. Starting and ending times were recorded for each bite event. A list of events with columns for event order, starting time and ending time was created for each grazing session. A database with all events (bites) and their corresponding sound records and characteristics were created. Sounds were formatted for the “seewave” package (Sueur et al., 2008) and imported into R (R core team, 2014). An R program on seewave was created to read, cut and measure the integral of the envelope, and the relative power in each of 44 frequency bands for each event.

General analysis

The effects of the SSH HM, actual SSH and the BkD of the superior strata were tested using analysis of variance and significant differences between the means were carried out by using the Tukey test at 5% probability.

The relationship of the three methods to estimate the BM and the SSH, was tested by the linear regressions (yij=a + bx + €ij) regressions, where: “yij_” is the dependent variable (BM), “a” is the intercept, “b” is the linear coefficient, “x” is the independent variable and “€ij” represent the experimental error. The maximum LogWorth (Log10 (P value) defined the optimal model. We also performed a decision tree model – hierarchical segmentation of data – to define the principal acoustics variables to predict bite mass for each of the three methods used to estimate the BM. All analyses, besides the sounds variables, were performed using JMP software (version 11; SAS Institute Inc., Cary, NC, USA).

Results

The sward characteristics in both forage species used are presented in Table 1. HM increased with SSH at both species. BkD of the top stratum was different between *L. multiflorum* and *C. dactylon*. Whereas for *L. multiflorum* BkD decreased with increasing SSH, in *C. dactylon* it increased with the SSH increasing.

Table 1. Sward characteristics of both forage species.

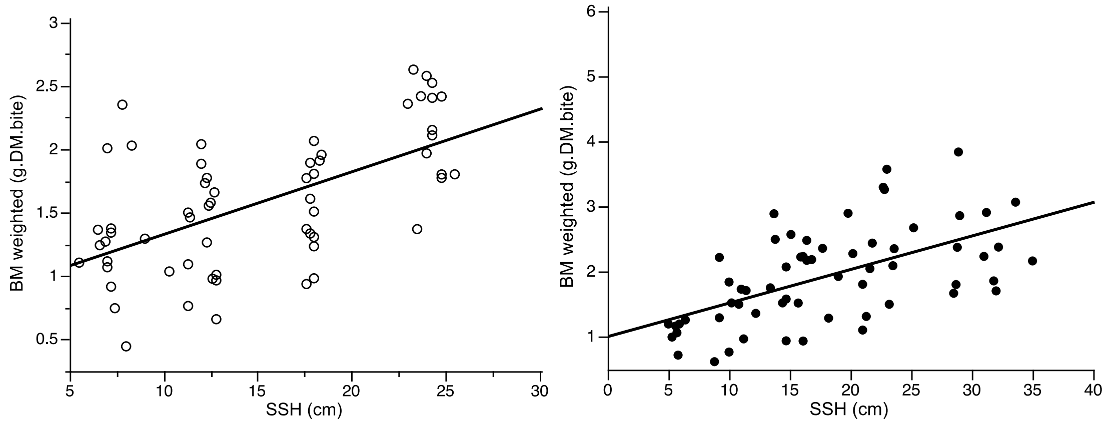
	Lolium multiflorum				
	6	12	18	24	P
HM (Kg.DM.ha^{-1})	919.9b±222.7	1852.4ab±197.0	1868.7ab±201.4	2384.5a±252.5	0.0004
SSH (cm)	7.43d±0.18	12.35c±0.16	17.67b±0.16	24.18a±0.20	<0.0001
BkD Superior Strata (g.m^{3})	2231.0a±157.2	1735.6b±139.0	1312.4b±142.2	988.7b±178.2	<0.0001
	Cynodon dactylon				
	12	19	26	33	
HM (Kg.DM.ha^{-1})	1990.9d±506.6	6127.3c±506.6	10843.8b±475.8	15004.7a±506.6	<0.0001
SSH (cm)	7.9d±0.75	14.3c±0.75	20.4b±0.71	29.1a±0.75	<0.0001
BkD of Superior Strata (g.m^{3})	1182.7b±209.1	3080.4a±209.1	3458.3a±196.5	3778.4a±209.1	<0.0001

The means values of BM obtained by the three different methods are presented in Table 2. We observe that heifers harvested larger bites in *C. dactylon* than in *L. multiflorum* swards.

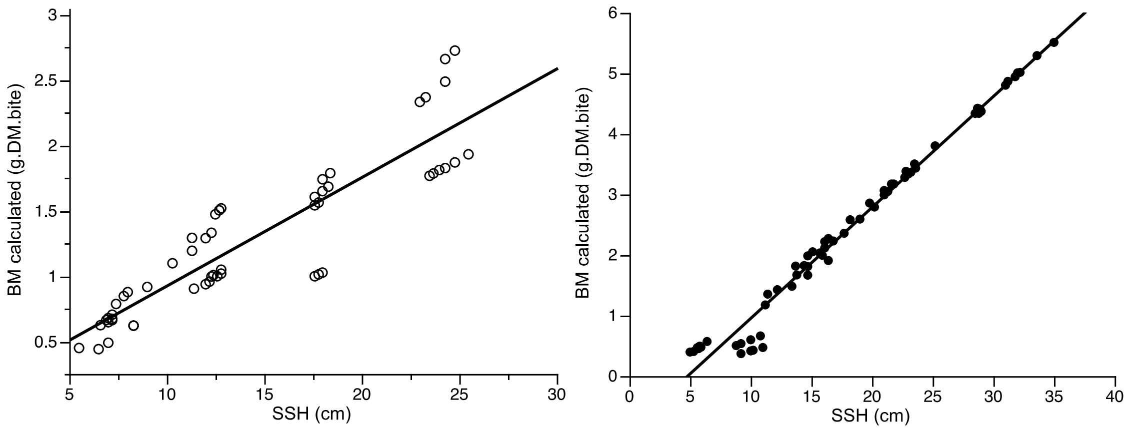
Table 2. Average and variances of BM obtained by three methods.

	Lolium multiflorum				
	6	12	18	24	P
Double Weight method (g. DM.bite^{-1})	1.20±0.092	1.60±0.090	1.96±0.092	2.37±0.115	<0.0001
Calculated method (g. DM.min^{-1})	0.69±0.074	1.28±0.065	1.70±0.067	2.13±0.084	<0.0001
Hand plucking method (g. DM. bite $^{-1}$)	0.29±0.067	0.57±0.059	0.78±0.061	0.68±0.076	<0.0001
	Cynodon dactylon				
	12	19	26	33	
Double Weight method (g.DM. bite $^{-1}$)	1.28±0.151	1.88±0.151	2.14±0.142	2.31±0.151	<0.0001
Calculated method (g. DM. bite $^{-1}$)	0.48±0.125	1.77±0.125	2.92±0.118	4.45±0.125	<0.0001
Hand plucking method (g.DM. bite $^{-1}$)	0.15±0.023	0.52±0.024	0.78±0.022	1.06±0.023	<0.0001

Double weight method



Calculated method



Hand Plucking method

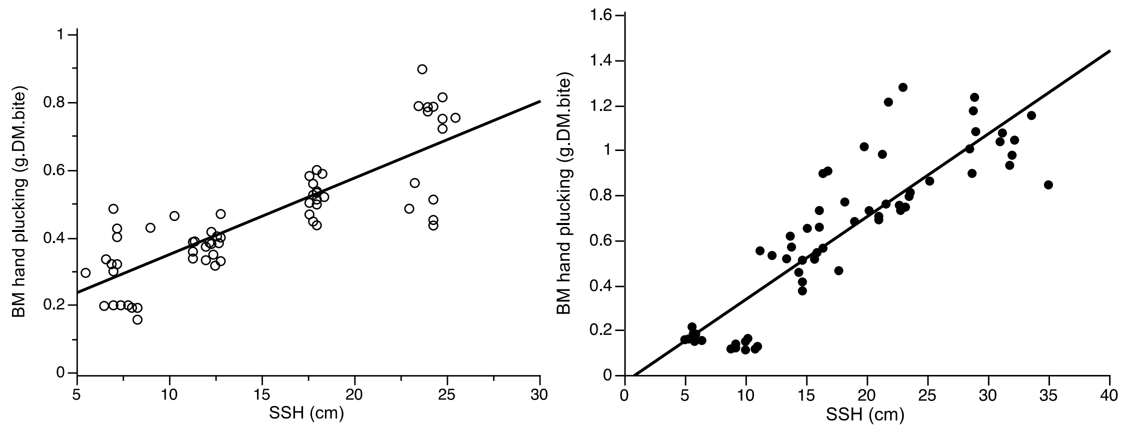


Figure 1. Relationship between average bite mass measured obtained by three methods on both forage species (*Lolium multiflorum* (o) – double weight method - y = 0.83 + 0.049x; P<0.0001; R^2=0.35; RMSE=0.429; calculated method - y = 0.092 + 0.083x; P<0.0001; R^2=0.79; RMSE=0.268 and hand plucking method - y = 0.122 + 0.02x;

P<0.0001; R^2=0.70; RMSE=0.095. *Cynodon dactylon* (●) double weight method - y = 1.01+ 0.05x; P<0.0001; R^2=0.33; RMSE=0.603; calculated method - y = -0.87 + 0.18x; P<0.0001; R^2=0.98; RMSE=0.211 and hand plucking method - y = -0.03 + 0.04x; P<0.0001; R^2=0.77; RMSE=0.169).

The BM obtained by the three methods presented a linear relationship with each other (Figure 2). However, there are some points in the *L. multiflorum* swards (treatment 12cm and 18cm), all in the same day, for which bite mass was much smaller in the hand plucking than other methods. This fact may be attributed to the low DM proportion in the sward on this day, because this was the first grazing session since the sward was sown.

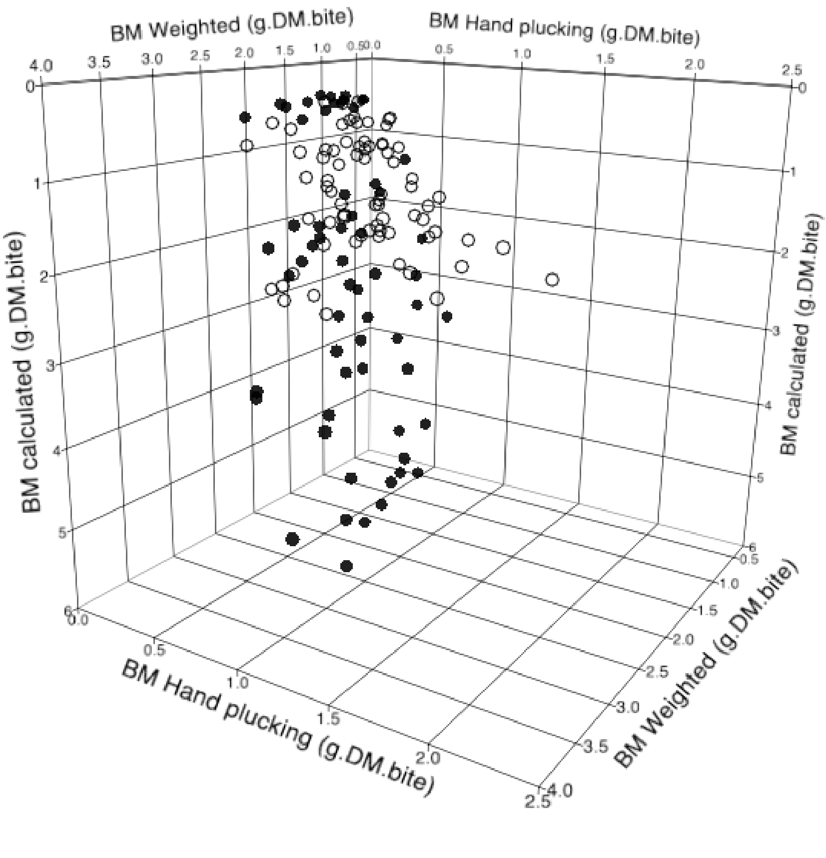


Figure 2. Relationship between average bite mass measured by microswards weights, hand plucking and calculated method (*L. multiflorum* (o) and *C. dactylon* (●)).

In Figure 3 we observe that is possible to estimate the BM from sound variables in *C. dactylon*. We compared the three methods used in this work to estimate the BM, and all of these methods showed a possible estimation of BM from sound variables for *C. dactylon* swards.

Calculated method Double weight method Hand-plucking method

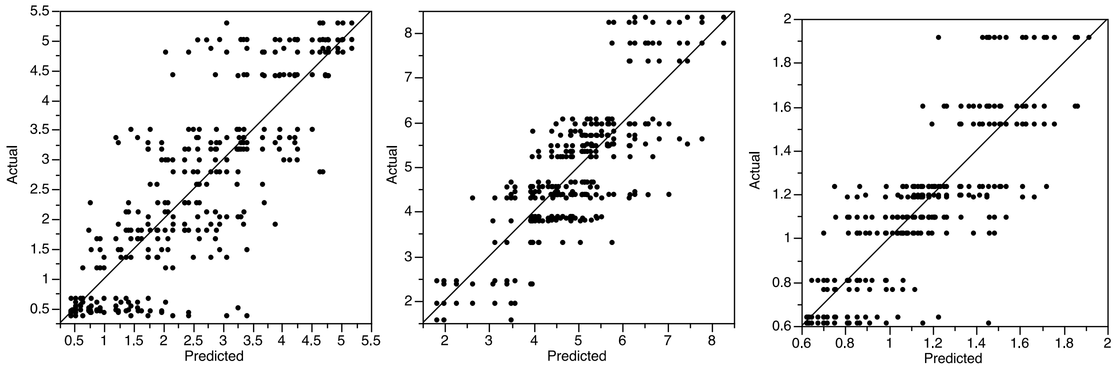


Figure 3: Actual values from the three methods used to estimate the BM versus predicted values from the sound variables in *C. dactylon* swards. Calculated method - R^2: 0.74; RMSE: 0.796; AICc: 1282.7; N: 462. Double weight method - R^2: 0.71; RMSE: 0.781; AICc: 1260.28; N: 462. Hand plucking method - R^2: 0.77; RMSE: 0.176; AICc: -111.47; N: 461.

Discussion

The values of BM found in this work, measured by double weight and calculated methods, were higher than other values of BM found in the literature. Fonseca et al. (2013) found a BM of 1.4 g DM on Sorghum bicolor swards grazed by the same heifers of this work, when they were 24±2month old and 306±56.7 kg of BW. Mezzalira et al. (2014), in turn, found a BM of 1.5 in *Avena sativa* swards grazed by the same heifers when it 45±2 months old and 349±20 kg live weight. The variables determining BM are bite depth, bite area and bulk density of the grazed stratum (Laca et al., 1992). As the bite area is less sensitive to changes in sward structure, the high values of BM observed, mainly on *C. dactylon* swards (Table 2) may be attributed to the high BkD on the superior strata in this species (Table 1). The increase of BkD observed when SSH was increased may be due to plants age, because, *C. dactylon* swards were implanted just four months. before the grazing tests, thus, on the stage of grazing tests were performed the plants were in stage of filling the space of the microswards, which provide the BkD increasing with SSH.

According to Figure 1, BM is highly related with SSH for both *C. dactylon* and *L. multiflorum* swards. We hypothesized that BM would be an asymptotic function of SSH. However, the results showed that linear relationships were adequate. This may be a result of the fact that SSH relative to animal BW was not sufficient to "saturate" the ability of heifers to harvest this forage. The structure of microswards presented high BkD, and, given that the plants were young and in early vegetative stages, this BkD was basically composed of leaves. It is known that animal prefer swards with large proportion of leaf and high BkD, because it enables greater ideal SSH que BM (Gregorini et al, 2009b;. Gregorini, 2012). Therefore, due to this optimal structure animals were able to take large BM with high SSH.

Considering the linear relation between all methods of estimation of BM (Figure 2), is possible to obtain a good estimation of BM by any method, and the choice of the ideal method depends of the protocol conditions.

Double weight method

The double weighing method is difficult to be adopted in several circumstances. This method can be used in experiments with microswards, however it is difficult to adopt in a free-grazing circumstances. The construction of microswards requires intensive labor, moreover, this technique requires some form of anchoring the plants if they are not to be uprooted (Orr et al., 2005). Alternatively, as in the case of the present work, the roots of plants may be allowed to penetrate an under-layer of growing medium, but this would require time. However this technique would have a low requirement for seeds, which may be in short supply early in a forage breeding program (Beerepoot & Agnew, 1997).

The double weight method also requires a high accuracy scale. Furthermore, it is necessary accuracy in the DM samples, as well, the accuracy in a weighing of the microswards, avoiding the occurrence of material losses between the double weights, because it can produce error in BM estimation. In this work, we found higher BM values in the double weight method, which can be explained, besides of the high BkD described above, by the high dry matter values, which could be produced by errors in weighing the green samples, or inaccurate scales, or may have occurred by loss of material not harvested by the animal between the first and the second weight of the microswards.

Calculated method

The BM calculated by the formula from Baumont et al. (2004), is product of: bite area, bite depth, and bulk density of the grazed stratum (50% of the SSH). This formula is considered a good estimator, however, this method requires the knowledge of some swards characteristics. According to Table 2, this formula has a tendency to overestimate BM for tall swards and underestimate for short swards, these were also observed by Baumont et al. (2004). This method can be used in many field conditions, however, it will always be an estimation, but it can be used if the researcher knows the swards characteristics with enough reliability. Some animal characteristics, such as bite area and bite depth, can be measured to make this method more reliable.

Hand plucking method

According to the results, it is possible to estimate the BM visually. However, BM estimated by the hand plucking method was smaller than observed in both double weight and calculated methods (Figure 1). This “underestimation” by hand plucking method may be attributed to the small, although, proportional samples by evaluator. Thus, this method could be improved, if the evaluator was more trained to gather greater samples in each bite type by hand. Some studies have often criticized hand plucking as a method of herbage intake estimation, suggesting that is subjective method (e.g., Gordon, 1995; Forbes, 2006). However, other studies (e.g., Bonnet et al., 2011, Wallis de Vries, 1995) suggest that this method is efficient to estimate the BM accurately. According to Bonnet et al. (2011), those concerns can be dismissed with observer training. These authors suggest that training of the observer is the most important variable influencing the hand plucking method to estimate the BM accurately. This method can be used in many protocols, however, as described above, is necessary to use observers trained and able to identify BM accurately. Besides the observers, the animal must be familiar with the presence of the observer; otherwise, the human presence can interfere in the animal behaviour.

Combining hand plucking with automated recording methods may produce a good data set and, consequently, can be very useful to understanding the grazing strategies (Bonnet et al., 2011). Thus, the hand plucking method combined with bioacustic method, which was tested in this work (described below), may be a good way to improve the research in ingestive behavior area.

Estimated BM from sound variables in C. dactylon microswards

Among recent innovations in the estimation of herbage intake, the bioacoustics has been proposed because it is a non-invasive, low cost and allows identification of the the animal's behaviors continuously without affecting the animal behavior. This method had been proposed by a significant number of publications (e.g. Laca Wallis & DeVries, 2000; Milone et al, 2009; Clapham et al, 2011; Galli et al, 2011; Trindade et al., 2011; Nadin et al, 2012). According to Figure 3, is possible to estimate the BM using the bioacustic in *C. dactylon* microswards; this result agrees with previous works (i.e. Galli et al, 2011; Laca & Wallis De Vries, 2000) which suggest that is possible to estimate the herbage intake using the acoustics characteristics. The advantage of this method is the possibility of use in many protocol conditions. Microphones can be placed in animal in free grazing condition, and the chance to estimate the herbage intake along the ingestive behavior is a good advance for research in grazing management, among the other areas of animal science.

Conclusion

The relationship between BM and SSH is linear within the SSHs studied. All methods to estimate the BM can be used in a particularly condition, although, the BM estimated from the bite types is smaller, when it is compared to the bite masses measured and calculated. The BM can be estimated by sound variables, i.e. bioacustic; however, it needs improvement to estimate BM and herbage intake in grazing condition.

Acknowledgments

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3.CAPITULO III

Acoustic discrimination of cattle bite types with multiple microphones[1]

[1]Artigo elaborado de acordo com as normas da revista Computers and Eletronics in Agriculture (Apêndice 2)

Acoustic discrimination of cattle bite types with multiple microphones

Abstract: The understanding of the factors that affects the ingestive behavior of grazing animals is very important to support research in animal science. This study aimed to determine the effect of microphone position ingestive sounds by grazing cattle. The main objective was to study the possibility of discriminating between bites and chew-bites, as well as among bite types previously defined whose weights can be estimated by hand-plucking. The experiment was carried out at the Experimental Station of Agronomy School, Federal University of Rio Grande do Sul, using *Cynodon dactylon* microswards. Four, Angus × Brahman, beef heifers (60 ± 2 month old, 395 ± 6.7 kg BW) were used. The treatments were four contrasting sward surface heights (SSH, 12, 19, 26 and 33cm), selected to create different structures and result in a ide range of bite mass. In each grazing session, one animal was allowed to graze a microsward until it started taking bites from the second grazing horizon. Each bite was observed from less than xxx m and its type was recorded. Bite mass was estimated by the hand plucking method. Grazing sounds were recorded with a digital recorder (Edirol R-09 24-bit recorder, Program Version 1.20, Roland Corporation), and an omni-directional lavalier microphone (Leson ML-70s), in WAV (Waveform Audio) file format. Three microphones were place on the animal, one on the forehead, one by the mouth and one on the back. Sounds were analysed using the “seewave” package in R, whereby a measure of total sound energy (envelope integral) and the proportion of sound energy in each of 44 frequency bands were calculated as predictors. Bite type was analysed by discriminant analysis with all sound variables as potential predictors. There was no diferences between sounds characteristics to discriminate the bite types, on the different positions of the microphones. However, the sound quality was better in forehead position facilitating the analysis by listening. The sound characteriscs were efficient to discriminate bite and chew bites, however the sound characteristics were no accurate enough to discriminate this kind of bite types previously defined on this experiment.

Keywords: Bioacustic, Single bite, Chew-bites, Bite mass.

1. Introduction

The understanding of the factors that affect the ingestive behavior of the grazing animals is very important to support the research in animal science as well as support grazing management. Since Penning et al. (1984) which develop an original system to recorder the ingestive behavior, several others systems have been developed. Brun et al. (1984) developed a microcomputer-based system, which consisted in a small rubber balloon placed at submandibular space and connected to a microphone. However, this system only informed time spent eating and ruminating, with no information about the number or types of jaw movements.

The initial development of bioacustic to study the ingestive behavior of domestic herbivores, is reported by Matsui and Okubo (1991), these authors developed a system based on a sensor connected to a digital recorder. This system gives the information about the time spent eating and ruminating, total number of jaw movements during these activities. However, this/that system did not allow discrimination between bites and chews, consequently, could not be used to obtain reliable information about bite mass. Luginbuhl et al. (1991), in other hand, developed a microcomputer-based incorporated to a microphone, this system allowed to describe the number of bites, by ‘listening’ the sound (2600-3000 Hz).

In the evolution of scientific research, Rutter et al. (1997) described other system able to be used in ingestive behavior studies, which was called IGER Behaviour Recorder. This system, developed by the IGER institute was widely used, for many studies about ingestive behavior (e.g. Fonseca et al., 2013; Orr et al., 2004). The data obtained by the IGER behavior recorder was analyzed by the Graze software, which gave us the total time spent in grazing, rumination and idling, as well the total jaw movements. The IGER system discriminated for the first time bites and chews.

These techniques were not wholly satisfactory, only some of that discriminates jaw movements, like IGER behavior recorder, but not all the richness of bite features was identified. For example, the the discrimination of compound jaw movements, just is possible using the bioacoustic technique (Laca et al., 1992; Laca and Wallis De Vries, 2000; Ungar et al., 2006). Therefore, these methodology needs to be improved, in aspects like: patterns of use, and analysis of the grazing sounds.

This study aimed to determine the microphone position effect on ingestive sounds by grazing cattle. The main objective was to study the possibility to discriminate bites and chew-bites in different positions, as well the capacity of the bioacustic to discriminate previously defined bite types.

2. Materials and methods

2.1 Animals and Procedures

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Federal University of Rio Grande do Sul and were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

2.2 Experimental site, animals, treatments and sward measurements

This study was carried out at the Agronomic Experimental Farm of the Federal University of Rio Grande do Sul, Brazil. Grazing sessions were conducted with four Angus × Brahman, beef heifers (60 ± 2 months old, 395 ± 6.7 kg BW) and *Cynodon dactylon* microswards. The *Cynodon dactylon* were planted in 84 boxes (microswards) each measuring 0.6 m X 0.4 m, 64 of these was used to the grazing session and the remaining was used to training the animals. The planting was done on November 2013, with seedling density of 5t/ha, which is, approximately 8 seedlings per box. All grazing sessions (described below) were performed in the afternoon in both experiments.

Thirty days before experiment, heifers and evaluators were trained to the experimental protocol with the remaining microswards. The heifers grazed a paddock containing *Cynodon dactylon* sward. Approximately 30 d before and during the experimental period, the animals were familiarized with observers, recording equipment and the experimental procedures, and remained in an adjacent paddock. Before each grazing session, heifers were fitted with a halter and a saddle, where microphones were attached in each position, described below.

To create different structures and different bite mass (BM) and consequently trying to achieve distinct bite sounds, the treatments were four levels of sward surface height (SSH) 12, 19, 26 and 33cm in *Cynodon dactylon* microsward. The treatments were randomly applied in a 4 × 4 Latin square design, with four tratments of SSH and four animals, each latin square was replicated four times, thus, each animal receveid a designated sequence of four treatments at the afternoons. The animals were observed in random order, and the sequence of treatments was randomized within animal with the restriction that each animal received a different sequence.

A sward stick was used to determine the sward surface height, were measured 10 points per microsward, pre- and post-grazing, in each grazing session. All herbage samples were oven dried (55°C over a period of least 72 h). A digital scale (model Bel Mark 720, 0.001 g of division) was used to measure the samples weights.

2.3 Grazing sessions

The four heifers remained grazing a free-grazing area of *C. dactylon* before the observations began. At the time of start the grazing sessions, the designated heifer was drawn from the group and the acoustic equipment was allocated at each position in the animal (described below). The heifer was led calmly towards the centre of the first microsward, so as to approach perpendicularly to the long axis of the patch. The microsward, used in the grazing session, was placed on the floor so that the soil surface was approximately 15 cm above the level at which the animals were standing. The microsward was offered to each animal and remained until he started to take bites in the second grazing horizon, and each bite was described according to a type predefined (bite tyoes were described below). Bites were determined visually, by the evaluator positioned to the side of the animal, and the bites were later checked against the listen the sounds and video track, when necessary.

2.4 Bite mass estimations

Specific coding grids (bite types - ga2, me2, rep) was developed to help the observer to categorize bites with regard to mass. These types were developed according to visually the way that the animal take each bite, thereby, ga2 is a big BM, when the animal used all the capacity of tongue to take the bite; me2 is smaller bite, when the animal do not used all the capacity of tongue to take the bite and rep is a bite when the animal used the area opened by the previous bite to take another one.

Prior to data collection, the observer was trained for 2 d. Training consisting of first recognizing the bites and classifying them according to the coding grids, and then, collect bite mass, by “hand plucking”, according to each bite type identified in each grazing session.

2.5 Sounds measurements

Grazing sounds were captured on the system consisted of a digital recorder (Edirol R-09 24-bit recorder, Program Version 1.20, Roland Corporation), and an omni-directional lavalier microphone (Leson ML-70s). Sound data was recorded onto a 2GB SD memory card (Sandisk Extreme III SDHC Card Sandisk Corporation) in the Edirol R-09. All recordings were made at 44.1 kHz sampling rate and 16-bit resolution, providing a nominal 20 kHz recording band, width and 96 dB dynamic range, and stored in the WAV (Waveform Audio) file format. Prior to each recording session, the recorder input level; sampling rate and bit resolution were set; the recorders were secured inside the plastic enclosure.

The microphones were allocated in three positions, namely:

Forehead: On this position the microphone was accommodated in an involucre of polystyrene expanded and remained in direct contact with the forehead of animal. On this position the sound was captured by animal bone and air.

Mouth: On this position the microphone was placed on the halter, such that the microphone was 7-10cm near the mouth of the animal and capturing the bite sound which travel by air.

Back: On this position the microphone was placed in a saddle on the back of the animal, such that the microphone was 80-100cm far from the mouth of the animal and capturing the bite sound which travel by air.

2.6 Statistical analysis

Audacity software for Mac (version 2.0.5, http://audacity.sourceforge.net), The stereo files was reduced to monaural files by extracting one channel. Records from different microphones were manually synchronized and labelled. Sounds were simultaneously segmented and classified "by hand" using the software Audacity. Starting time, ending time and type was recorded for each event (b- bite; cb – chew-bite; bite types: ga2, me2, rep). A list of events with columns for event order, type, starting time and ending time was created for each grazing session. A database with all events (bites) and their corresponding sound records and characteristics were created. Sounds were formatted for the “seewave” package (Sueur et al., 2008) and imported into R (R core team, 2014). An R program on seewave was created to read, cut and measure the integral of the envelope, and the relative power in each of 40 frequency bands for each event.

To separate bite types and to discriminate bites from chew bites, we performed discriminant analysis based on the linear method. The effects of the SSH for BM, HM, actual SSH were tested using analysis of variance and significant differences between the means were carried out by using the Tukey test at 5% probability. All analyses, besides the sounds variables, were performed using JMP software (version 11; SAS Institute Inc., Cary, NC, USA).

3. Results

Different structures and bite masses were achieved by the gradient of SSH in *C. dactylon* swards (Table 1).

Table 1. Herbage mass (HM), sward surface height (SSH) and bite mass (BM) estimated by hand plucking method on *C. dactylon* microswards.

	Treatments			
	12	19	26	33
Herbage mass (Kg.DM.ha-1)	1990.9d*	6127.3c	10843.8b	15004.7a
Actual SSH (cm)	7.9d	14.3c	20.4b	29.1a
Hand plucking BM (g.DM.min^{-1})	0.15d	0.52c	0.78b	1.06a

*Means followed by the different letters differ significantly to the level of 5% probability.

Figure 1 shows the average spectrograms for each bite type recorded by each microfone position. As expected, the mouth position recorded greater proportion of the sound energy in high frequencies, however, this did not result in a better ability to discriminate bite types.

3.1 Chew bites discrimination

Table 2 presents the sound variables used to discriminate between bite and chew-bites. The analysis is presented of two ways, at the first one was permitted the analyses use the Bite length, and after this variables was excluded of analyses. these variables selected by stepwise selection.

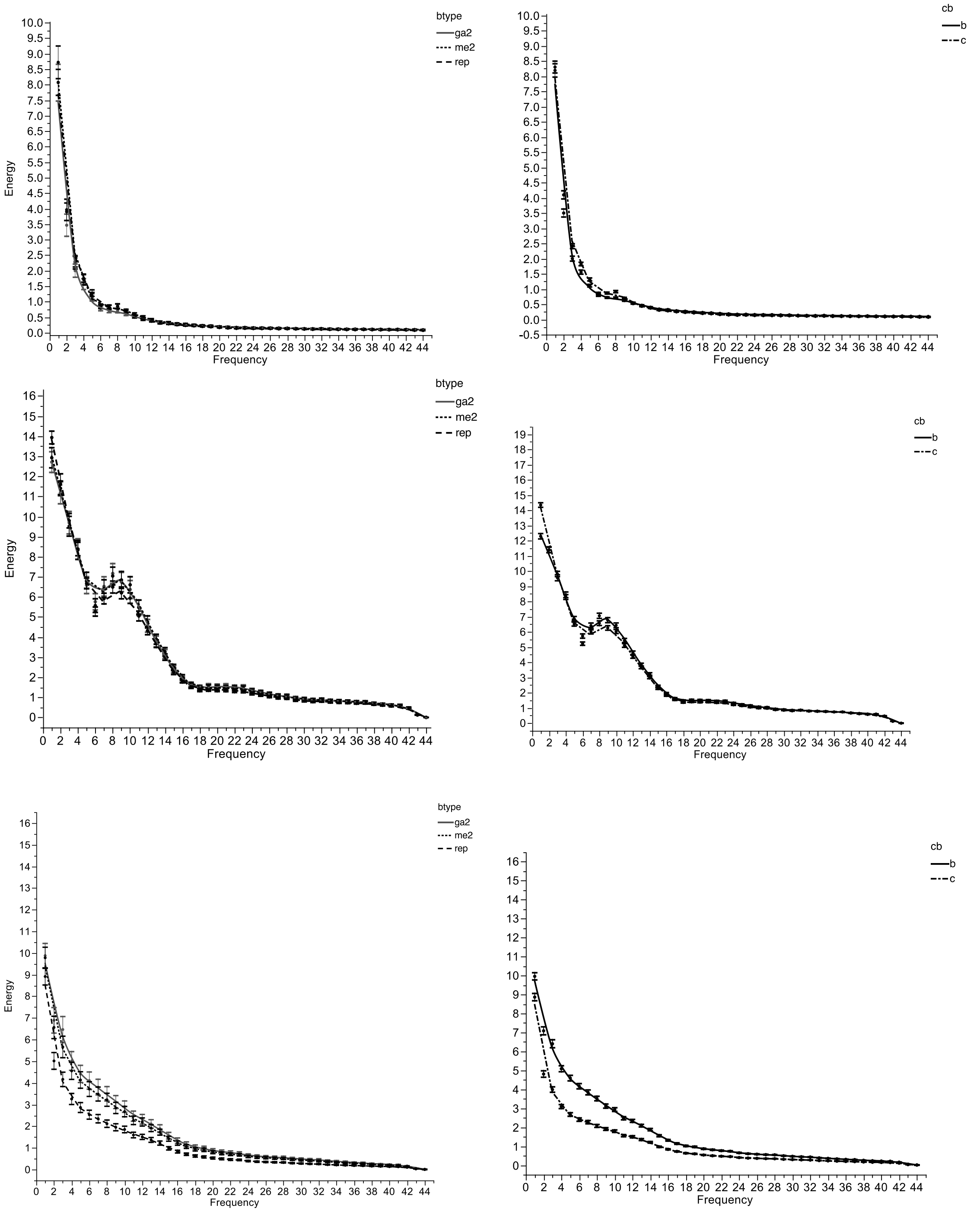


Figure 1 Energy in each bite frequency, according to bite types (i.e: previously defined on left and single or compound on right) in each position: Forehead; Mouth and Back, in the top, meddle and bottom, respectively.

Table 2. Variables included in each microphone position to Bites and Chew Bites discrimination:

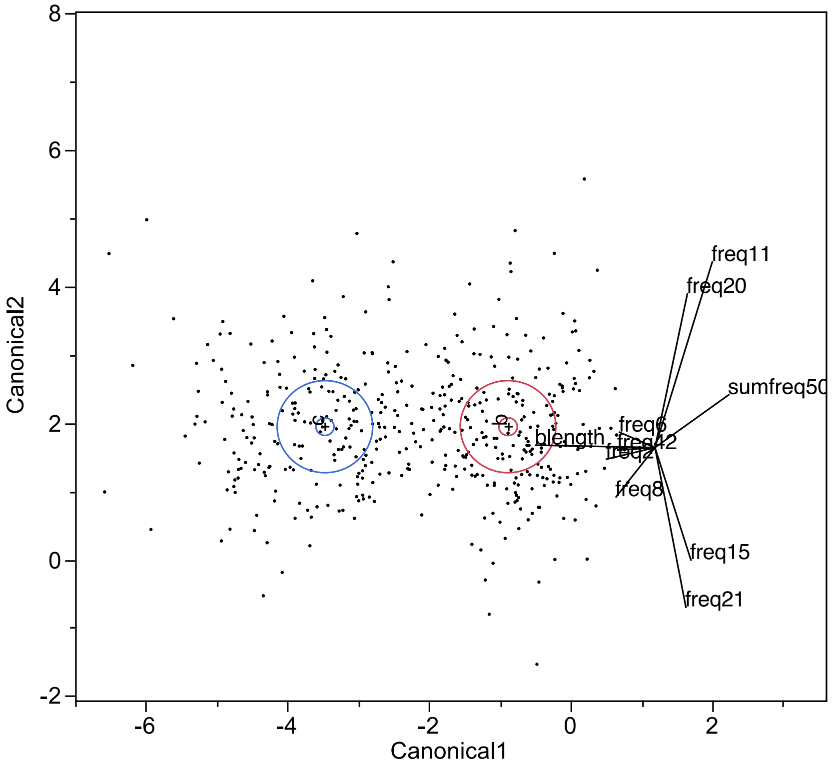
Bite length included			Bite length excluded		
Forehead	**Mouth**	**Back**	**Forehead**	**Mouth**	**Back**
Bite length	Bite length	Bite length	freq1	freq1	freq1
freq2	freq1	freq1	freq2	freq2	freq4
freq6	freq6	freq3	freq5	freq3	freq5
freq8	freq17	freq5	freq7	freq5	freq8
freq11	freq18	freq12	freq10	freq6	freq12
freq15	freq21	freq18	freq14	freq14	freq20
freq20	freq23	freq25	freq21	freq17	freq25
freq21	freq37	freq38	freq32	freq24	freq30
freq42	freq41	freq44	freq41	freq37	freq41
Envelope	Envelope	Envelope	Envelope	Envelope	freq44

On table 3 and Fig. 2 are presented the numbers of bites and chew-bites and the canonical plot, in which were identified visually and separate according to sound variables by discriminant analysis. The misclassified proportion was 8.1; 9.16 and 8.32% to Forehead, Mouth and Back, respectively, when bite length is included. When bite length is excluded of analyses the misclassified proportion was: 20.26, 24.09 and 21.54 to Forehead, Mouth and Back, respectively.

Table 3. Actual (Rows) by Predicted (Columns) to Bites (b) and Chew Bites (cb) discrimination:

	Bite length included						Bite length excluded					
	Forehead		**Mouth**		**Back**		**Forehead**		**Mouth**		**Back**	
	b	**cb**	**b**	**cb**	**b**	**cb**	**b**	**cb**	**b**	**cb**	**b**	**cb**
b	218	13	208	23	206	25	185	46	169	62	157	74
cb	25	213	20	218	14	224	49	189	51	187	27	211

Bite length included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freq15
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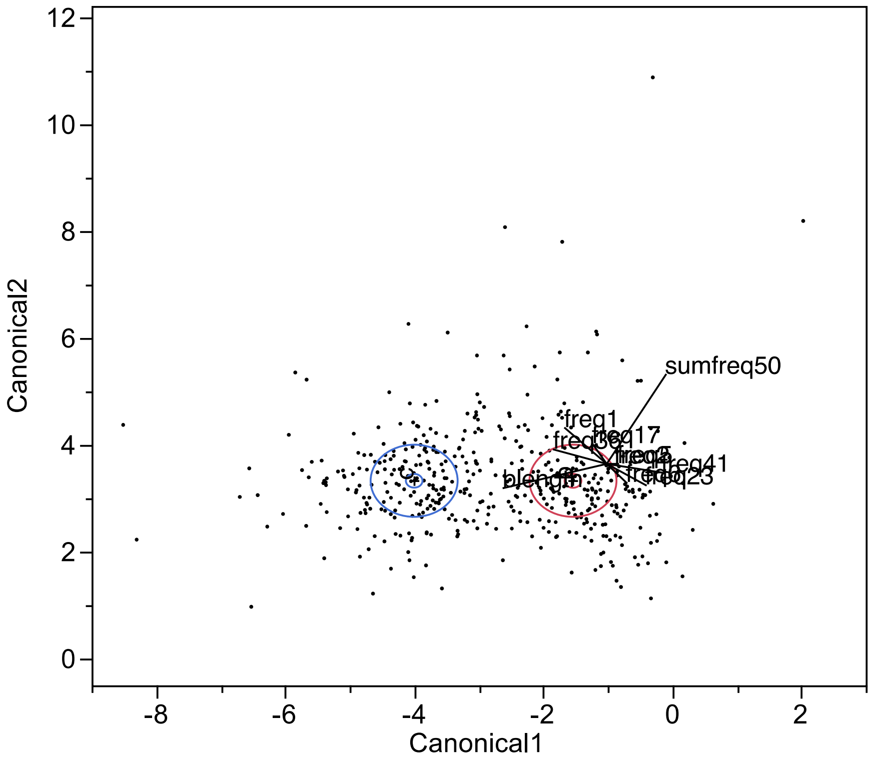
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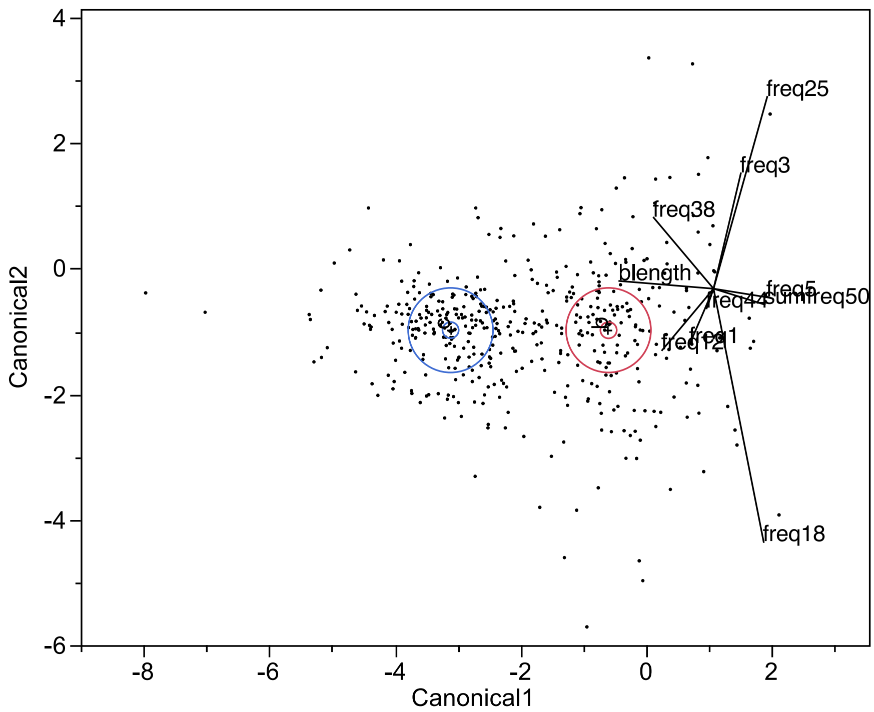
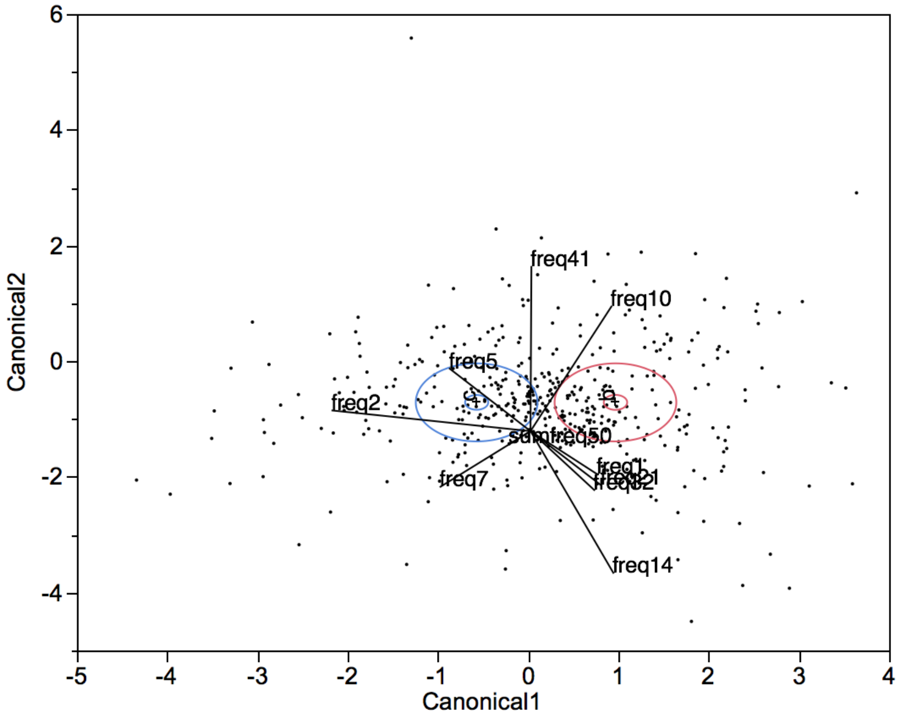


Bite length excluded



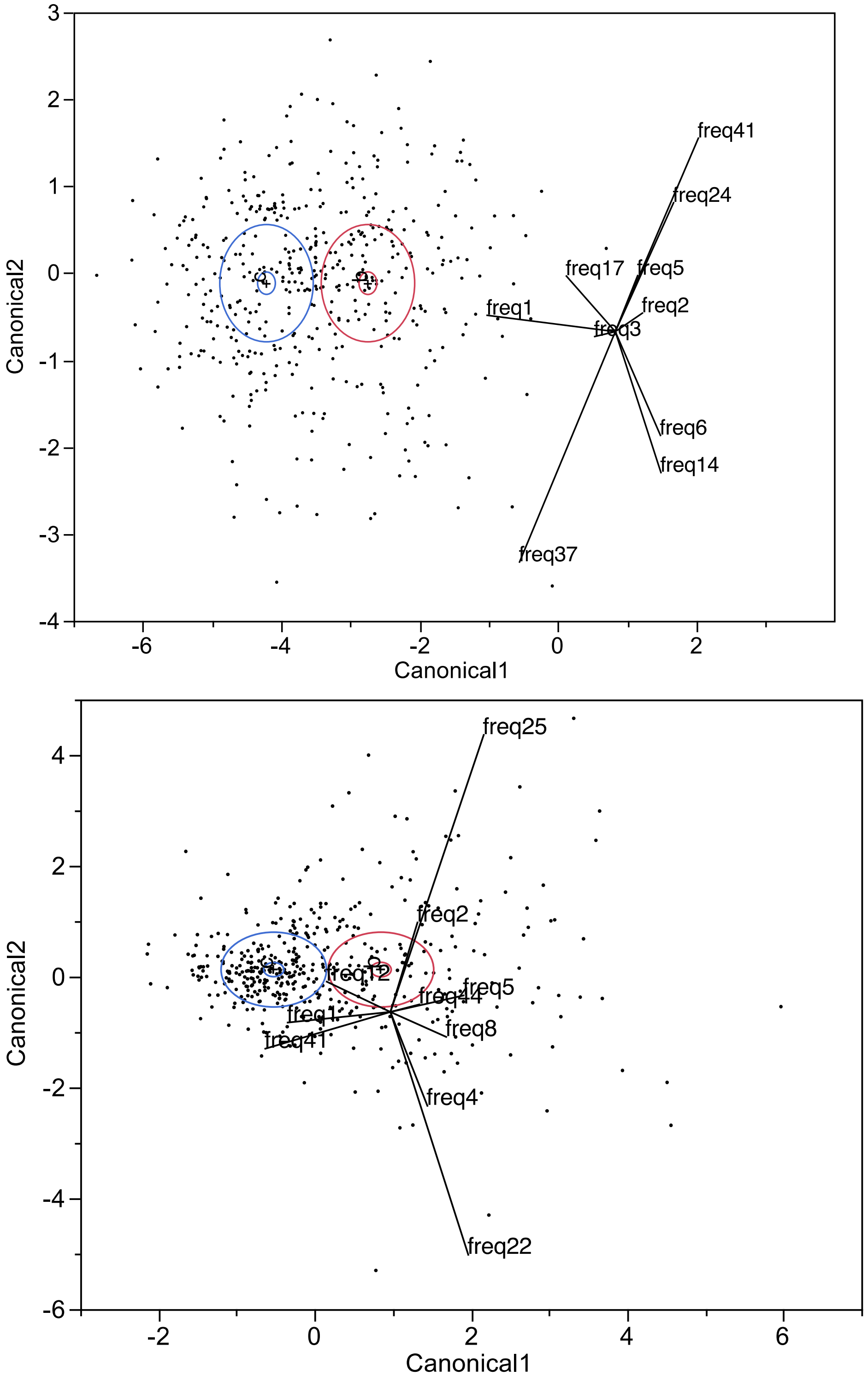


Figure 2. Canonical plot of single and compound discrimination for *Cynodon dactylon* microswards grazed by beef heifers, with bite length included and excluded in Forehead, Mouth and Back repectively (bites – red; and chew bites – blue).

3.2 Bite type discrimination:

On Table 4 is presented the sound variables used to discriminate between bite types, which were previously defined.

Table 4. Variables included in each microphone position to Bite types discrimination.

Forehead	**Mouth**	**Back**
Bite length	Bite length	Bite length
freq5	freq1	freq2
freq7	Freq8	freq19
freq8	Freq10	Freq29
freq16	Freq16	freq33
freq22	Freq17	freq42
freq36	Freq21	freq43
freq40	freq34	freq44
Envelope	Envelope	Envelope
SSH	SSH	SSH

The values of BM are presented on Table 5, according each bite type previously defined, and the SSH treatments. The BM differ significantly with the SSH and with bite types.

Table 5. Bite mass in each SSH and each bite type for *C. dactylon* sward

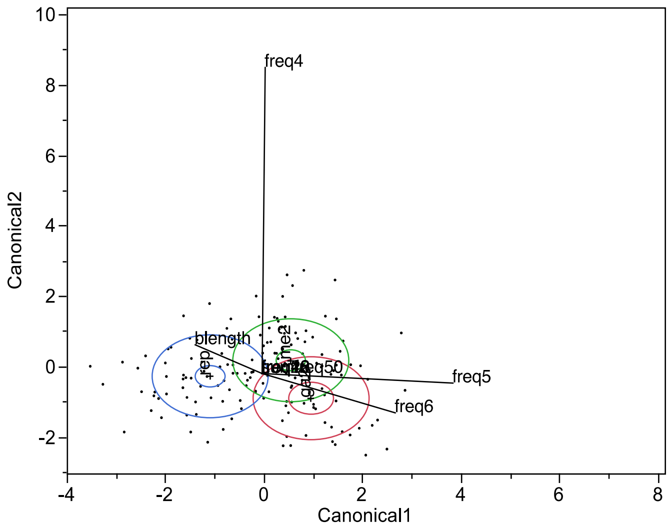
Bite type	**SSH**			
	12	**19**	**26**	**33**
ga2	0.93Da*	1.45Ca	1.65Ba	1.92Aa
me2	0.73Db	1.02Cb	1.27Bb	1.52Ab
rep	0.81Dc	1.06Cb	1.15Bc	1.23Ac

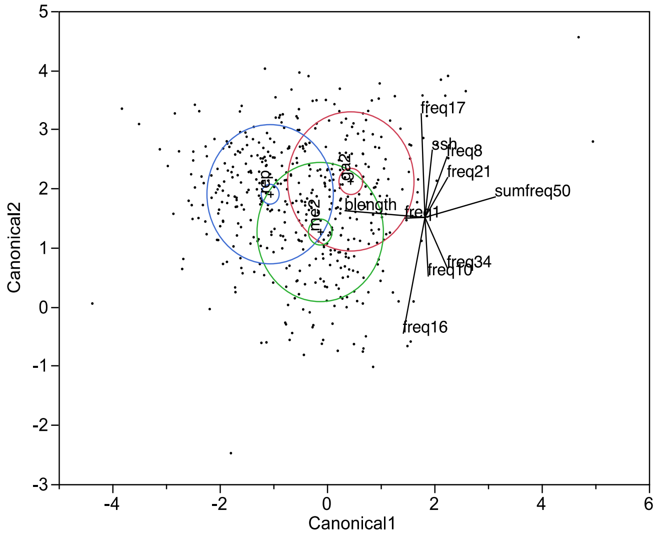
*Means followed by the same lower case letters in a column and capital letters on the lines do not differ significantly to the level of 5% probability.

On Table 6 and Fig. 3 are presented the numbers and graphic representation of each bite type, which were identified visually and separate according to sound variables by discriminant analysis. The misclassified proportion to discriminate the bite types previously defined were: 38.9, 39.1 and 37.4% to forehead, mouth and back, respectively.

Table 6. Actual (Rows) by Predicted (Columns) to Bite types discrimination

	Forehead			**Mouth**			**Back**		
	ga2	**me2**	**rep**	**ga2**	**me2**	**rep**	**ga2**	**me2**	**rep**
ga2	73	24	25	75	24	23	67	30	24
me2	30	61	34	23	68	33	23	62	39
rep	35	32	148	35	42	138	18	38	159





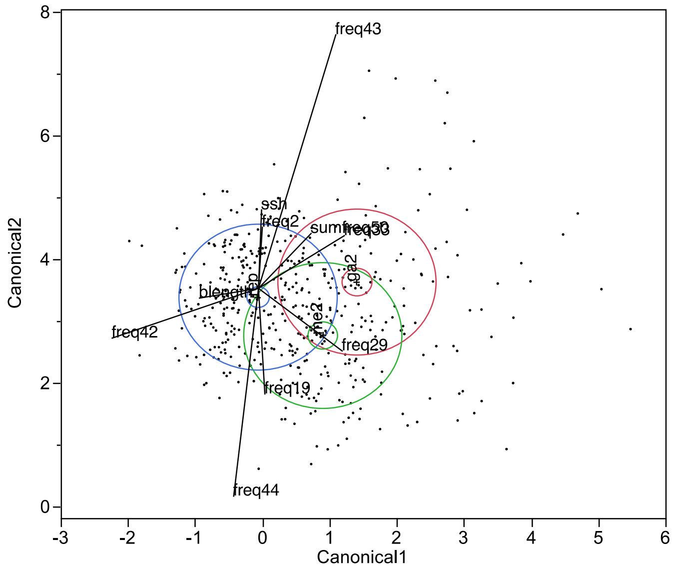


Figure 3. Canonical plot of bite types discrimination for Cynodon dactylon microswards grazed by beef heifers, in Forehead, Mouth and Back repectively (Bite types: Ga2 – red; me2 – green; rep – blue).

4. Discussion

Ingestive behaviour reflects the integration of the abundance and quality of the grazing environment with the animal's choices, which is important to support grazing management. There are many techniques to evaluate ingestive behaviour, such as Ethosys, IGER behaviour recorders, APEC and recently bioacustics, which is presented in this paper. Sounds records contain a lot of information which can be gathered in a way that does not interfere with grazing behavior and that may lend itself to automated analysis (Laca & Wallis De Vries, 2000). Furthermore, acoustics characteristics allows accurate counts of chew and bite events (Laca & Wallis De Vries, 2000; Galli et al., 2006; 2011; Clapham et al., 2011). Therefore, this paper present ways to standardize the bioacustic methodology. We intend in this work to show the possibility to discriminate single bites and chew bites as well as bites types previously defined and identified, in *Cynodon dactylon* microswards grazed by beef heifers by using information contained in the sounds of grazing.

Several previous studies have shown the possibility to identify grazing events based on sounds characteristics (e.g. Laca & Wallis De Vries, 2000; Galli et al., 2006; 2011; Clapham et al., 2011). Various microphone positions have been used. In some studies the microphone is located in the forehead of the animal (Galli et al., 2006, 2011) where bite sounds recorded are transmitted through the air and the animal's bone. On the other hand, in some studies the microphone is located near the mouth (Clapham et al., 2011); in this case, sounds recorded are transmitted mostly through the air. This poses the question of whether one postion is better than the other. According to Laca & Wallis De Vries (2000), the use of a single microphone may results in a recording with a variety of sounds unrelated to the chewing and biting of forage that hinder interpretation.

According to the results, there are no diferences in frequency characteristics according to microphone position and the possibility of classification of bite types (Both for: bite and chew bites, as previously defined bite types). Therefore, it was easy to discriminate bites and chew-bite by listening the sounds of the microphone positioned on the animals forehead. On mouth position there are more wealth details in bite energy, at the median frequencies which can help on automated bite recognition (Figure 1).

4.1 Chew-bites discrimination

An important advantage of the acoustic method is that in addition to measure number of bites and chews, this method allows to discriminate the compound jaw movements (chew-bites) (Galli et al., 2011). The ‘‘chew–bites’’ happen when the herbage already in the mouth is chewed as the jaws close to grip and sever fresh herbage. This phenomenon was reported for several animal species, such as: Girafes (Ginnett and Demment, 1995), sheep (Rutter et al., 2002) and heifers (Ungar et al, 2006, laca et al., 1994). Chew-bites allow the animals to reduce the total number of jaw movements per bite without reducing the number of chews per bite (Galli et al, 2011). Thereby, to know the proportion of chew-bites in behaviour studies is necessary to make consistentes conclusions and improve the undertanding of plant-animal relationship.

According to the results, is possible to discriminate between bites and chews-bites. The bite length is the most importante variable that determine this discrimination (Table 3). The differences in bite length between bites and chew-bites are clearly shown, visually, on the spectrogram (Fig. 4). Using the bite length the misclasified proportion on discriminant analysis was 8.102%, 9.16% and 8.32% in Forehead, Mouth and Back respectively, while when bite length was excluded of analysis, the proportion goes to 20.26%, 24,09% and 21.54% in Forehead, Mouth and Back repectively.

(a) (b)

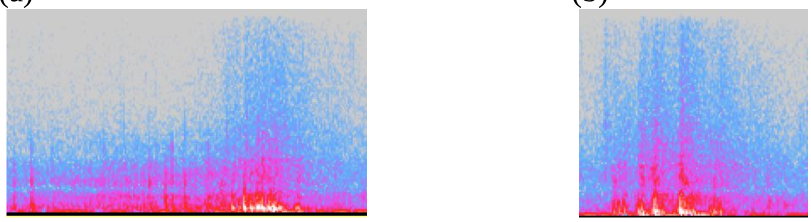


Fig. 4. Representative figure of compound bite (chew-bite) (a) and single bite (b) for *Cynodon dactylon* microswards grazed by beef heifers.

4.2 Bite types discrimination

Discriminate different types of jaw movements is important in order to estimate bite mass accurately (Rutter et al., 1997). However, according to results of the present study, the bite types identified and determined visually by the evaluators, was not discriminate by the sounds variables (Table 6). Probably, there is no high accuracy in sounds characteristics to discriminate the previously determined bite types. This may be is due to the small difference in bite mass, and in grazed structure between these bite types (Table 5).

5. Implications

There are no diferences between sound characteristics in different positions of microphone. However, to identify events by listening the bites sound was easier in the forehead position. The sound characteristics were efficient to discriminate between bite and chew bites, however this afirmation is not true to discriminate the others bite types proposed on this paper.

Ultimately, we can state that bioacustic had richness of information, and can be used to discriminate bite events, as well, some bite types, single or compound, however is necessary improve the research in this area. This improvement must be in order to get more information about acoustics characteristics, and these informations must be in an automated form. In other words, the research in bioacustic for ingestive behaviour, urgently need an automated way to analyse the bites acoustic characteristics.

6. Acknowledgments

This work was supported by grants from the Brazilian National Council for Scientific and Technological Development (CNPq).

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4. CAPITULO IV

4.1 Considerações finais

4.1 CONSIDERAÇÕES FINAIS

4.1.1 Perspectivas futuras de curto prazo

Como já é de conhecimento dos pesquisadores que trabalham na área de produção animal, e como foi descrito no decorrer deste trabalho, um dos principais gargalos na pesquisa científica é a medição do consumo de forragem pelos animais à pasto. Esta variável é de fundamental importância na grande maioria das pesquisas realizadas. E embora haja vários avanços em diversas metodologias, como uso de marcadores, hand plucking, etc, ainda não há um consenso de qual a melhor meotologia que proporcione uma avaliação mais precisa.

Neste contexto, o método acústico tem se mostrado um método promissor para analisar o comportamento ingestivo, e como demonstrado neste trabalho e em outros citados no decorrer do mesmo, têm a possibilidade de estimar o nível de ingestão de forragem por ruminantes domésticos.

No capitulo II desta tese, foram abordadas metodologias de estimar a massa do bocado. Dentre todas as metodologias avaliadas pode-se afirmar que, dependendo de cada protocolo e objetivo de estudo elas podem ser utilizadas. Por fim, neste mesmo capitulo abordou-se o uso da bioacústica como uma metodologia emergente para este fim, e constatou-se que “sim” há grandes possibilidades de esta vir a ser uma metodologia à ser amplamente utilizada.

Entre os entraves para a plena adoção da bioacústica, no entanto, a automatização as anáises dos eventos ainda e o principal deles. A identificação automática de eventos acústicos pode exigir extenso estudo preliminar para estabelecer modelos para processos de reconhecimento, análise acústica detalhadas e métodos computacionais complexos (Sueur et al. 2008) e isto é o que se pretende fazer na sequencia do presente trabalho.

Neste trabalho foi verificado que é possível identificar visualmente onde ficam os bocados e através de suas características sonoras, analisadas após esta identificação, demostram que há diferenças acústicas entre bocados de um “tipo” ou de outro, e que de acordo com outros trabalhos pode-se diferir, inclusive, as características de bocados de diferentes massa.

No capitulo III desta tese, foi demostrado que a variáveis acústicas são capazes de discriminar tipos de bocados. Esta diferenciação está mais evidente entre bocados simples e compostos, e não tão evidente e precisa para tipos de bocados previamente definidos. Esta falta de acurácea na distinção pode ser por falta de softwares adequados para este fim, o que não permitiu a exploração de todas as características que se vislumbra com os dados acústicos.

Durante a realização do período de doutorado sanduiche da autora foi realizado alguns avanços na automatização das análises de eventos, no entanto este avanço, apesar de ser importante ainda não é o suficiente para fornecer uma perspectiva mais concreta de análise automátizada. E esta automatização exige, ainda, muitos meses de trabalho de programação no software statistico R com o pacote seewave. Esta fase, inclusive, esta prevista para acontecer no período de pos doutorado da autora. Espera-se que em poucos anos tenhamos esta metodologia com um protocolo de uso e análises estabelecidos, testados e prontos para serem utilizados nas pesquisas de comportamento ingestivo e consumo por animais em pastejo.

4.1.2 Possibilidades futuras de longo prazo

A perspectiva futura de longo prazo com a bioacústica, é que esta técnica permita o avanço na chamada “Pecuária de precisão”, Quando falamos de “Agricultura de Precisão” logo se imagina a busca pela padronização da área, no intuito de eliminar a heterogeneidade, trabalhando com maquinários que fazem adubações, irrigações, dentre outras técnicas de manejo visando homogeneizar a área em questão. No caso da pecuária, no entanto, o objetivo deve ser outro, ou seja, a lógica reside na questão de que o manejador deve conhecer os processos envolvidos no pastejo, e aprender com estes processos, para criar ambientes favoráveis ao pastejo pelos animais (Carvalho et al., 2009).

Nesta ótica, pode surgir o questionamento: Onde a bioacústica entra neste contexto? Ela entra, primeiramente, no entendimento dos processos. Como descrito no decorrer deste trabalho, a bioacústica é altamente promissora como metodologia de avaliação do comportamento ingestivo dos animais em pastejo. Quando o manejador compreende o comportamento animal, e entende o porquê de cada decisão tomada ou não pelo animal, ele se torna capaz de inferir sobre ambientes pastoris adequados e manejá-los adequadamente.

Além do monitoramente do comportamento ingestivo, a bioacústica é uma ferramenta altamente promissora à longo prazo. Esta ferramente pode ser utilizada como uma ferramenta de campo, onde pode ser possível conhecer como, onde, quanto e o quê os animais estão consumindo, e isto pode auxiliar em decisões práticas de manejo, como: mudanças de piquetes, suplementação, etc. Além disso, esta técnica pode ser vislumbrada associada ao monitoramento remoto, com o mesmo objetivo, facilitando ainda mais a ação do manejador.

4.1.3 Contextualizacao do trabalho e perspectivas do GPEP na sequência da bioacústica

O primeiro contato do GPEP (Grupo de Pesquisa Ecologia do pastejo) com a técnica da bioacústica aconteceu no *International Symposium on the Nutrition of Herbivores* no Texas. Vários desenvolvimentos ocorreram até que primeira tese de doutorado com bioacústica no GPEP fosse defendida por Dr. Júlio Kuhn Da Trindade, onde se obteve registros sonoros com a utilização de gravadores digitais de voz simples em diferentes protocolos experimentais. As conclusões desta primeira tese permitiram confirmar a possibilidade de uso da bioacústica para quantificar o tempo diário das atividades de pastejo e ruminação, uma vez que possuem padrões de registros sonoros bastante distintos e facilmente discriminados em softwares de áudio (Da Trindade et al., 2011).

Portanto a presente tese é a segunda na área da bioacústica desenvolvida pelo GPEP. Neste trabalho, como descrito nos materiais e métodos dos artigos, foram adotados o uso de gravadores e microfones que captam e registram o som em ampla frequência (0-22kHz) e que os armazena no formato WAV, o que de acordo com Clapham et al., 2011 irá facilitar a diferenciação automática de movimentos mandibulares. Na sequência da presente tese o GPEP pretende, por meio de projetos que já estão aprovados pelo CNPq e envolvem a utilização dos dos dados obtidos nesta tese, dar sequencia no desenvolvimento da automatização da bioacústica pelo pacote seewave do software R.

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6. APÊNDICES

Apêndice 1. Normas em que foi escrito o capitulo II
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Spelling: The Journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

Units: All measurements must be given in SI or SI-derived units.

Abbreviations: Abbreviations should be used sparingly – only where they ease the reader's task by reducing repetition of long, technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only.

(CONTINUAÇÃO) Apêndice 1. Normas em que foi escrito o capitulo IV

Scientific names: Upon its first use in the title, abstract and text, the common name of a species should be followed by the scientific name (genus, species and authority) in parentheses. However, for well-known species, the scientific name may be omitted from the article title. If no common name exists in English, the scientific name should be used only.

PARTS OF THE MANUSCRIPT

The length of an article (including references, tables and appendices) should not exceed 6 pages for research papers, 3 pages for short reports and 12 pages for review papers.

Manuscripts should be presented in the following order: (i) title page, (ii) abstract and key words, (iii) text, (iv) acknowledgments, (v) references, (vi) appendices, (vii) figure legends, (viii) tables (each table complete with title and footnotes) and (ix) figures. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Title page

The title page should contain (i) the title of the paper, (ii) the full names of the authors and (iii) the addresses of the institutions at which the work was carried out together with (iv) the full postal and email address, plus facsimile and telephone numbers, of the author to whom correspondence about the manuscript should be sent. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

The title should be short and informative. Do not use abbreviations in the title. A short running title (less than 40 characters) should also be provided.

Abstract and key words

All articles must have a brief unstructured abstract that states in 200-300 words (100-150 words for short report) or fewer the purpose, basic procedures, main findings and principal conclusions of the study. The abstract should not contain abbreviations or references.

Five or fewer key words (three or fewer for short report) should be supplied (for the purposes of indexing) below the abstract in alphabetical order.

Text

Authors should use the following subheadings to divide the sections of their manuscript: Introduction, Materials and Methods, Results and Discussion.

Statistics

Whenever possible, researchers should consult a statistician before designing an experiment and when analyzing results to avoid inappropriate descriptions.

(CONTINUAÇÃO) Apêndice 1. Normas em que foi escrito o capitulo IV

Describe statistical methods with enough detail to enable the reader to verify the reported results. Indicate the computer program used, if any.

Acknowledgments

The source of financial grants and other funding must be acknowledged, including a frank declaration of the authors' industrial links and affiliations. The contribution of colleagues or institutions should also be acknowledged. Personal thanks and thanks to anonymous reviewers are not appropriate.

References

The Harvard (author, date) system of referencing is used (examples are given below). In the text give the author's name followed by the year in parentheses: Smith (2000). If there are two authors use 'and': Smith and Jones (2001); (Smith and Jones 2001). When reference is made to a work by three or more authors, the first name followed by *et al.* should be used: MacDonald *et al.*(2002). In the reference list, references should be listed in alphabetical order. In the reference list, cite the names of all authors when there are six or fewer; when seven or more, list the first three followed by *et al.* Do not use *ibid.* or *op cit.* Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (e.g. Smith A, unpublished data). All citations mentioned in the text, tables or figures must be listed in the reference list.

Authors are responsible for the accuracy of the references.

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Hirata M, Sato R, Ogura S (2002) Effects of progressive grazing of a pasture on the spatial distributions of herbage mass and utilization by cattle: a preliminary study. *Ecol Res* 17: 381–393.

Online article not yet published in an issue

An online article that has not yet been published in an issue (therefore has no volume, issue or page numbers) can be cited by its Digital Object Identifier (DOI). The DOI will remain valid and allow an article to be tracked even after its allocation to an issue.

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Hirosaki S (1978) Basic experimental layout. In: *Applied Statistical Handbook* (Ed Okuno T), Yokendo, Tokyo, 202–229.

Use 'Eds' when the book is edited by two or more editors: (Eds Okuno T,

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FAO (1999) Guidelines on social analysis for rural area development planning. Agricultural policy support service, FAO, Rome, available from URL:http://www.fao.org/tc/Tca/pubs/tmap34/tmap34.htm [cited 2 November 2004].

Appendices

These should be placed at the end of the paper, numbered in Roman numerals and referred to in the text. If written by a person other than the author of the main text, the writer's name should be included below the title.

Tables

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COMPUTERS AND ELECTRONICS IN AGRICULTURE

An International Journal

AUTHOR INFORMATION PACK

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INTRODUCTION

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5.3 *For books*
Peart, R.M., Brooks, R.C. (Eds.), 1992. Analysis of Agricultural Energy Systems. Energy in World agriculture, 5. Elsevier, Amsterdam.
5.4 *For multi-author books* Price, D.R., Chen, T.H., Peart, R.M. 1992. Acknowledge-based decision system for control of waste heat for a greenhouse-aquaculture complex. In: Peart, R.M., Brooks, R.C. (Eds.), Analysis of Agricultural Energy Systems. Energy in World Agriculture, 5. Elsevier, Amsterdam, pp. 33-46.
5.5 For unpublished reports, departmental notes, etc. Deshazer, J.A., Moran, P., Onyango, C.M., Schofield, C.P., 1988. Imaging systems to improve stockmanship in pig production. Div. Note 1549, AFRC Institute of Engineering Research, Silsoe, UK.
6. Do not abbreviate the titles of periodicals mentioned in the list of references; alternatively use the *International List of Periodical Title Word Abbreviations.*
7. In the case of publications in any language other than English, the original title is to be retained. However, the titles of publications in non-Latin alphabets should be transliterated, and a notation such as "(in Russian)" or "(in Greek with English abstract)" should be added.
8. Work accepted for publication but not yet published should be referred to as "in press".
9. References concerning unpublished data and "personal communications" should not be cited in the reference list buy may be mentioned in the text.

Apêndice 3. Fotos dos experimentos e materiais utilizados.

Foto 1: Equipamentos (gravadores de voz digital e microfones) adquiridos pelo projeto e utilizados nas avaliações. Créditos - L. Fonseca.

Lolium multiflorum ***Cynodon dactylon***

Foto 2: Detalhes das bandejas com micropastos das duas especies utilizadas nos ensaios. Créditos - L. Fonseca.

(CONTINUAÇÃO) Apêndice 3. Fotos dos experimentos e materiais utilizados.

Foto 3: Fotos ilustrando o momento das desfolhações nos testes de pastejo, ao lado percebe-se a avaliadora (aluna de doutorado Lidiane Fonseca) registrando em seu gravador o "grid" de bocados que ela estava observando. Créditos - J. K. Da Trindade.

Foto 4: Sequência da realização dos testes de pastejo, da esquerda para a direita. Pesava-se a caixa em balança configurada para alta precisão; que então era fornecida ao animal e permitido tomar em torno de 10-15 bocados contados e identificados com códigos de "tipos" de bocados (detalhes do método em Agreil & Meuret (2004) e Bonnet et al. (2011); por fim, pesava-se novamente para ter o valor médio da massa do bocado. Créditos - J. K. Da Trindade.

7. VITA

Lidiane Fonseca nasceu em 13 de março de 1986 na cidade de Verê/PR, filha de Lidia e Garibaldi da Fonseca. Cursou o ensino fundamental entre 1993 e 2000. E o ensino médio entre 2001 e 2003. Em 2004 ingressou na UTFPR – Pato Branco, onde em 2008 graduou-se engenheira agrônoma. Durante o curso de agronomia desenvolveu atividades de iniciação científica, atuando de atividades laboratoriais e de campo nas áreas de Botânica, Bovinocultura e Forragicultura. Foi bolsista da PET em vários projetos de extensão universitária. Atuou nas áreas de manejo de espécies forrageiras anuais, bovinocultura de leite, dentre outras. Em 2009 iniciou o curso de Mestrado junto ao Programa de Pós-Graduação em Zootecnia da UFRGS, na área de concentração Plantas Forrageiras, como bolsista CNPq. Em Março de 2011 obteve o grau de Mestre em zootecnia. Em Abril de 2011 ingressou no curso de Doutorado junto ao Programa de Pós-Graduação em Zootecnia da UFRGS, na área de concentração Plantas Forrageiras, como bolsista CNPq. Entre Junho de 2013 e Maio de 2014 realizou seu doutorado Sanduíche, como bolsista do CNPq, na Universidade da Califórnia, Davis.